Recovery Plan for the killifishes of Bermuda
(*Fundulus bermudae & Fundulus relictus*)

Government of Bermuda
Ministry of Home Affairs
Department of Environment and Natural Resources

Recovery Plan for the killifishes of Bermuda
(*Fundulus bermudae & Fundulus relictus*)

Prepared in Accordance with the Bermuda Protected Species Act 2003

Author

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Cover photo: male and female *Fundulus relictus* in Bartram's Pond
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"To conserve and restore Bermuda's natural heritage"

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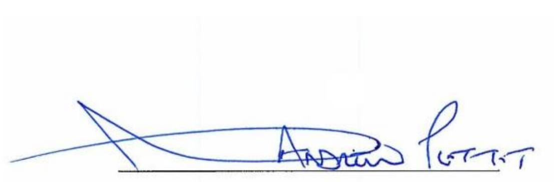

10th August 2020

Andrew Pettit
Director
Department of Environment and Natural Resources
Bermuda Government

Date

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EXECUTIVE SUMMARY

This is an updated plan and super cedes the Recovery Plan for Killifish in Bermuda published in 2011.

Current Species Status:

This recovery plan addresses the need for actions to conserve two endemic species of killifish, *Fundulus bermudae* and *Fundulus relictus*, on Bermuda. Both species are still listed as Endangered (EN, B1a, biii) as per IUCN criteria, under the Protected Species Act 2003 but have benefitted from a series of successful translocations between 2008 and 2012 which has increased their abundance as well as area of occupancy. Because of these translocation efforts, each species (assuming there are three) now inhabits at least three separate ponds (one of the criterion for recovery listed on 2011 killifish recovery plan). The most recent population assessments (2012) revealed that five of the nine sub-populations studied had estimated abundances greater than 5,000 individuals (another criterion listed in the 2011 recovery plan). Population estimates ranged from 1,012 to 69,373 individuals per pond. Mangrove Lake contained the largest population of *F. bermudae* (approximately 69,373 fish in 2011) and Lover's Lake the largest population of *F. relictus* (approximately 7,907 in 2011). It is worth noting that there is possibly a third endemic *Fundulus* species found within the western ponds on Bermuda, but this awaits genetic confirmation. The most recent genetic investigation (2012) the notion that the killifish in Evan's Pond and Warwick Pond are an evolutionary significant unit within the *F. bermudae / F. relictus* lineage and should be treated accordingly from a management perspective.

Habitat Requirements and Threats:

Killifishes are highly adaptable and capable of living in water that has wide ranges in temperature and salinity. In Bermuda, they are currently found in 15 ponds. Historical distribution was much greater and included various marshes, canals, muddy bays and coastal mangrove communities. It is thought that the principle factor which led to their current limited distribution is loss of habitat and fragmentation of wetlands. Pollution of ponds has also contributed to the decline in habitat quality, because ponds and marshes were historically used as garbage disposal sites. These ponds continue to be negatively affected by storm water run-off from roads, aerial deposition, and contaminated groundwater sources which carry petroleum hydrocarbons, polycyclic aromatic hydrocarbons, trace metals, and pharmaceutical metabolites. Predation and collection for home aquaria also threaten Bermuda's killifishes.

Recovery Objective:

The main goal of this plan remains the same as that of the 2011 plan; 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 fully resolved (i.e. when the total number of species, or at least the degree of intra-population genetic sub-structuring, is known).
- An updated assessment has been undertaken for all extant sub-populations.
- All potential habitats suitable for growth, reproduction and survival are identified, assessed, and restored.
- Each species of *Fundulus* inhabits at least three separate ponds.
- Each species inhabits at least one pond that has been officially designated as 'Critical Habitat'.
- Long-term, sustainable levels of killifish are reached in each pond that has been designated as 'Critical Habitat' (i.e. population >5,000).

Actions Needed:

1. Resolve the phylogenetic relationships between the existing sub-populations.
2. Update the population assessments.
3. Identify and restore wetland habitats suitable for killifish introduction.
4. Increase abundance and expand area of occupancy through translocation and aquaculture (if deemed necessary).
5. Designate Lover's Lake as "Critical Habitat' for *Fundulus relictus* and Mangrove Lake and Seymour's Pond as "Critical Habitat' for *Fundulus bermudae*.

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. Developing budgets for each action are the responsibility of the leading party as outlined in the work plan.

Date of Recovery:

Meeting these recovery objectives is largely dependent on the availability of suitable habitat. Down listing will be considered following 10 years of implementation (2030), 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 29% of the extant endemic ichthyofauna of Bermuda (Smith-Vaniz and Collette, 2013) and up to 100% of the ichthyofauna inhabiting some of the inland pond environments (M.O. pers. obs.). Bermuda's killifishes are thought to have originated through one or more colonization events from eastern North American coastal populations of *Fundulus heteroclitus*, most likely from the state of Georgia (Able and Felley, 1988; Grady et al., 2001; Whitehead, 2010).

A dearth of information regarding the health and status of these *Fundulus* sub-populations prompted investigations into their biology and ecology (Outerbridge et al., 2007a & 2007b; Copeland, 2013). However; there have been few opportunities for natural range increase, due in great part to the discontinuity of Bermuda's wetlands and restriction in habitat availability. The fragmentation of the wetland habitat in Bermuda also makes these species vulnerable to human impacts. Due to these unresolved threats, both species of killifish are listed under the Protected Species Act (2003) as Endangered.

This recovery plan discusses conservation efforts for Bermuda's killifishes, summarizing current knowledge about their taxonomy, distribution, habitat requirements, biology and threats. The plan recommends the resolution of the genetic make-up of the species for all ponds, as there is some uncertainty regarding the existence of a third *Fundulus* species. Recovery depends on the availability of suitable habitat, hence the restoration of selected ponds is a priority in this plan. Finally, in order to ensure long-term sustainability on Bermuda, an increase in the area of occupancy for each species, as well as in overall abundance is deemed possible through translocation and captive breeding.

Update on conservation actions listed under the 2011 Recovery Plan:

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

Between 2008 and 2012 a study attempted to resolve the phylogenetic relationships between the various extant sub-populations of Bermuda's killifishes and to compare them with killifishes living in North and South Carolina. A diverse set of genetic markers, which included both mitochondrial (D-loop) and nuclear (microsatellite loci and loci within the Major Histocompatibility Complex) genomes, were analyzed to detect if hybridization had occurred and to determine if any evolutionary or conservation significant units exist on Bermuda. DNA was successfully extracted from 101 tissue samples obtained from seven Bermuda sub-populations; Lover's Lake (n=20), West Walsingham (n=10), East Walsingham (n=21), Trott's Pond (n=10), Mangrove Lake (n=20), Warwick Pond (n=10), and Evan's Pond (n=10). Preliminary Bayesian analysis indicated that the killifishes inhabiting Warwick Pond and Evan's Pond should be grouped together in a clade separate from the other sub-populations on Bermuda (Lai and Cohen, pers. comm.). Furthermore, early results for the microsatellites showed that the Bermuda samples had lower diversity compared to samples collected from North and South Carolina, which provided compelling evidence supporting the theory that a bottlenecking event had occurred in the past for Bermuda's killifishes (A. Lai and S. Cohen, pers. comm.). Another attempt to clarify the genetic make-up of Bermuda's killifishes occurred in 2013 when an ichthyologist from the USA was given fresh samples for a genetic fingerprinting and molecular dating investigation. 120 samples were obtained from six sub-populations which were extant at the time; Wind Reach Pond (the offspring from Warwick Pond; n=20), Evan's Pond (n=20), Mangrove Lake (n=20), Trott's Pond (n=20), Blue Hole Bird Pond (n=20), and Cooper's Island Pond (n=20). The results of this investigation are still pending.

2. Protect species and pond habitat through legislation.

Both species are protected under the Protected Species Act, however the Protected Species Order may need to be amended if a third island endemic is described in the future. A number of ponds inhabited by Bermuda killifishes are protected because they are situated within a park or nature reserve but no pond has been officially designated as 'Critical Habitat'. This mechanism exists through the Protected Species Act which states:

"Without prejudice to section 28 of the Development and Planning Act 1974, the Minister may by order designate as a protected area any critical terrestrial or marine habitat essential for the protection of a specified protected species and specify the location and boundaries of the area and the order may impose such prohibitions or restrictions on activities within the area as the Minister may consider necessary for the protection of that species. The Minister shall cause a map of each protected area to be published in the Gazette and deposited with the Director of Environment and Natural Resources for public inspection. Where a protected area is privately owned, the Minister may enter into an agreement with the owner for the protection and management of the habitat of a specified species. An agreement under subsection (3) may restrict or regulate the development or use of land within the protected area and the Minister may enforce an agreement against the owner and his successors in title as a restrictive covenant.

In the case of a critical marine habitat the order may impose restrictions—

prohibiting the mooring of a vessel;

prohibiting the anchoring of a vessel;
imposing speed limits on marine traffic; and
prohibiting or restricting the movement of marine traffic within the protected area.
In the case of a critical terrestrial habitat the order may impose restrictions—
prohibiting or restricting entry to the protected area; and

with respect to activities within the protected area.

Notice of intention

Where the Minister proposes to make an order under section 6 (designating an area to be a protected area), he shall, by publication of a notice in the Gazette, set out the details of the proposal and, in the case of an order under section 6, the location and boundaries of the protected area, and the restrictions proposed to be imposed on activities within that area. The Minister shall cause a copy of the notice under subsection (1) to be deposited with the Director of Environment and Natural Resources for public inspection. The notice under subsection (1) shall invite representations from the public and, in particular, from landowners whose property may be affected. No order shall be made under section 5, section 5A or section 6 until 30 days have elapsed since the publication of the notice required by subsection (1)."

3. Identification and restoration of wetland habitats suitable for killifish introduction.

All relatively large freshwater, brackish water, and marine ponds have been listed in Table 3 of the appendix. Where known, the characteristics thought to affect fish survival were collated including salinity, temperature, area, and levels of environmental pollution. The data came from a variety of sources. Temperatures and salinities were reported by Thomas et al. (1991), Outerbridge (2014), and from unpublished data. The pollution data was collected by the Bermuda Amphibian Project between 1997 and 2019. Pond areas were calculated in ArcGIS 9.0 using a 2012 digitized aerial orthophotograph of Bermuda. Information gaps in Table 3 should be filled in order for the ponds to be prioritized in reference to killifish habitation.

In 2014 a collaborative study involving the Bermuda Zoological Society, Fort Environmental Laboratories and the Bermuda Government was initiated to apply simple remediation techniques (e.g. aeration) to a select number of ponds. Exposure studies under controlled conditions have shown that the reduction of polycyclic aromatic hydrocabons in pond sediment has a positive effect on fish reproductive effort (D. Fort, pers. comm.), therefore the sediment in select ponds across Bermuda were subjected to continuous aeration for a 12 month period. Significant decreases were observed in the total levels of detectable PAHs within the benthic sediment (J. Bacon, pers. comm.). This project is still ongoing at the time of writing.

Organizations like the Bermuda Audubon Society and the Bermuda National Trust continue to undertake restoration projects within various nature reserves across the islands, which includes the creation of new ponds. The latest examples were the enlargement of Seymour's Pond in 2011 and the excavation of Eve's Pond in early 2020. These restoration projects provide a good opportunity for killifish range expansion.

4. Enhancing population numbers through captive breeding.

Ex-situ populations currently exist in Austria and the United Kingdom. In January 2011 four adult male and four adult female *F. bermudae* were collected from the Wind Reach Pond and sent to the Vienna Zoological Gardens in Austria for captive breeding and ex-situ conservation. Those individuals produced 90 offspring by January 2012 (A. Lamboj, pers. comm.). In August 2012 ten adult male and ten adult female *F. relictus* were collected from the Cooper's Island pond and sent to the Vienna Zoological Gardens. They began breeding later that same year (A. Lamboj, pers. comm.). The London Zoo received *F. bermudae* from the Vienna Zoological Gardens in 2013 for husbandry and display purposes, and the Chester Zoo received *F. bermudae* in 2016. Successful breeding was reported from both institutions (A. Lamboj and G. Garcia, pers. comm.). These off-island captive populations have not contributed to local population enhancement, however they have value as a safe-guard against future local extinction events.

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

Between 2008 and 2012, nine new sub-populations of killifishes were created on Bermuda using 2,492 fish (see Table 2 in Section F). All of the ponds were man-made and the donor fish came from the closest extant populations. Surveys in 2017 confirmed that six of the stocked ponds contained killifishes, but it is not understood why the translocated killifishes failed to survive in the other ponds.

6. Develop research programmes on reproduction of wild populations.

Research on wild populations has not occurred since Outerbridge et al. (2007b) reported on the seasonal breeding periodicity of the killifish in Mangrove Lake.

B. Taxonomy and description of species

Class: Osteichthyes
Order: Cyprinododontiformes (topminnows & carps)
Family: Fundulidae
Genus: *Fundulus*
Species: *bermudae*
relictus
Common name: Bermuda killifish, mangrove minnow, mangrove mullet

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 whose colonization of the islands occurred thousands of years prior to human habitation (Smith-Vaniz et al., 1999). *Fundulus bermudae* was first described in 1874 by Albert Günther, who named it in reference to its origin. 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 (Fig. 1). 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 but they diminish in appearance in both sexes over time. 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 (Fig. 2). They also possess a sheath of tissue on the anterior edge of the anal fin that functions as an oviduct during egg laying (Able and Hata, 1984).

Able and Felley (1988) described *F. relictus* (Figs. 3 and 4) from a single pond location in St. George Parish (Lover's Lake). This species is believed to be a relict form that was formerly more widely distributed throughout Bermuda. *Fundulus relictus* can be distinguished from *F. bermudae* via 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 may occur on Bermuda (Smith-Vaniz et al., 1999), and it is currently assumed that no pond contains mixed species. 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" (Grady et al., 2001). This view has been subsequently supported by others (A. Lai and S. Cohen, pers. comm.).

Figure 1. Mature male *Fundulus bermudae*

Figure 2. Mature female *Fundulus bermudae*

Figure 3. Mature male *Fundulus relictus*

Figure 4. Mature female *Fundulus relictus*

C. Current Status

Global Distribution

Wild populations of both species are only found on Bermuda; however there are captive populations established in the United Kingdom and Austria.

Local Distribution

At the time of writing, Bermuda's killifishes were collectively found in 15 ponds totaling approximately 22 acres (Fig. 5). *Fundulus relictus* is known only from three ponds in St. George's Parish; Lover's Lake, Bartram's Pond, and Cooper's Island Pond. *Fundulus bermudae* inhabits Mangrove Lake, Trott's Pond, the east and west Walsingham Ponds, Blue Hole Bird Pond, Shelly Bay Pond, Warwick Pond, Seymour's Pond, Riddell's Bay Pond, Sea Swept Farm Pond, Evan's Pond, and the pond in the Madagascar exhibit at the Bermuda Aquarium Museum and Zoo (BAMZ). Some sub-populations are original; others were artificially created. Table 2 in Section F provides a summary of the known killifish transfer histories among the various ponds. The appendix contains more detailed information about the different ponds inhabited by Bermuda's killifishes.

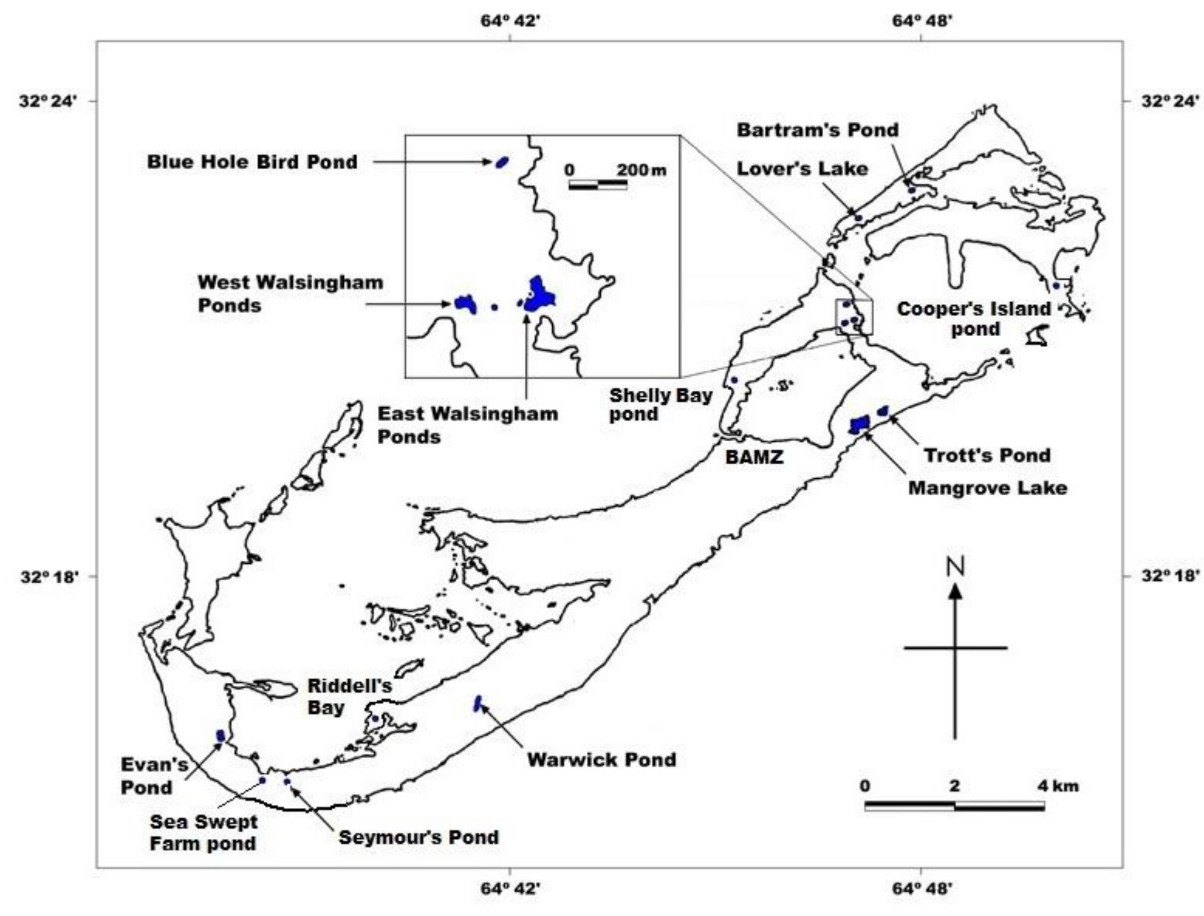

Figure 5. Map showing the 2017 distribution of Bermuda's endemic killifishes (Adapted from Thomas, 2002)

Species Protection

Neither *F. bermudae* nor *F. relictus* are subject to any international protective measures; however both are locally protected under the Bermuda Protected Species Act (2003) which classifies them as being Endangered (EN, B1a, biii as per IUCN criteria). This Act considers it an offence to willfully destroy, damage, remove or obstruct killifish habitat, as well as the taking, importing, exporting, selling, purchasing, or transporting of killifish (or any other listed protected species). Offenders are liable to a fine of up to $15,000 or one year imprisonment.

Habitat Protection

Most of the ponds (86%) which killifish inhabit occur in either nature reserves or parks and are therefore protected under the Bermuda National Parks Act (1986). Furthermore, ponds (and the fish within them) located on lands owned by the Bermuda National Trust are provided protection through the Bermuda National Trust (Open Spaces and Property) Regulations (1975). Lover's Lake and Warwick Pond are also designated as Ramsar sites, meaning they are considered to be wetlands of international importance.

D. Ecology

Habitat Requirements

Killifishes are extremely hardy. They live in salinities ranging from freshwater to full-strength salt water and withstand temperatures from almost freezing to 58°C (Griffith, 1974; Radtke, 1979). Another survival mechanism proving the robust nature of killifish is the ability to temporarily 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).

Fundulus heteroclitus, also known as the salt marsh killifish, mummichog, or mudminnow, inhabits coastal marshes, mudflats, river estuaries, tidal creeks, salt marshes, and lagoons in its native range along the eastern coastline of North America (Lee et al., 1980; Smith-Vaniz et al., 1999). Bermuda's killifishes, on the other hand, are presently only found within ponds (both natural and man-made), although there were historical populations in some inland marshes and coastal mangrove communities (Smith-Vaniz et al., 1999). Natural ponds on Bermuda vary both in size and in structure. Nearly all of the saline ponds date back in formation to the Holocene era (approximately 10,000 years ago.) The primary factor influencing salinity is the size and location of the underground connections each pond has with the ocean. Pond size and the nature of these connections influence the hydrographic characteristics of each pond. The sporadic addition of freshwater into these ponds from rainfall also influences salinity. Bermuda's marine ponds generally have a rich biota. Species richness increases with increasing physical stability and diversity of habitat, thus ponds having submerged rock substrata and an abundant submerged mangrove root community along the periphery of the pond 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, thus ponds close to the sea with relatively large connections have a higher flushing rate, narrower ranges of salinity and temperature and therefore provide a more stable environment than do ponds located 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. Salinity stratification can occur in poorly mixed ponds or where the connection to the sea is in the deepest part, although this phenomenon is unlikely to occur in very shallow ponds. Due to their small physical size and accumulated sediments, Bermuda's landlocked marine ponds are usually quite shallow, averaging depths of only 1.8 m. Thomas et al., (1991) described the physical characteristics of the six largest anchialine ponds on Bermuda (Mangrove Lake, Trott's Pond, Spittal Pond, Evan's Pond, Walsingham Pond, and Lover's Lake) after studying them over a ten year period. Most possess a single connection to the ocean. Surface salinities ranged from 6.5 to 42.5 practical salinity units (psu) and the temperatures varied from 15 to 37.5°C.

Biological Factors

Bermuda's marine ponds all have deep deposits of highly organic sediments. Surface run-off from surrounding land transports particulate matter and plant nutrients into the ponds. Fringing mangrove trees (*Rhizophora mangle, Avicennia germinans,* and *Conocarpus erectus*) are a common feature of marine ponds. These trees constantly drop leaves that slowly decompose, forming a highly organic detritus on the pond bottom that enhances the base of the food web. 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 that in open water marine habitats (Thomas and Logan, 1992). Competition, herbivory and predation are also generally less severe in ponds, thus favouring the growth and continued existence of some species that are rare or non-existent elsewhere on Bermuda (e.g. killifishes). 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 found on the pond bottom and in the water column. Studies indicate that they are opportunistic and omnivorous. Stomach content analysis has shown that the killifish inhabiting Mangrove Lake eat filamentous green algae and plant material, molluscs, crustaceans and insects (Rand, 1981). Outerbridge et al., (2007a) have shown that the size structure of Bermuda's various killifish sub-populations do not appear to be substantially different from *F. heteroclitus* populations along the east coast North America (Kneib, 1976) and in South West Spain (Fernandez-Delgado, 1989). In fact both maximum and mean sizes in all of Bermuda's extant sub-populations were larger than those reported by Kneib (1976) and Fernandez-Delgado (1989). Bermuda's killifishes average 6.1 cm total length (TL) but are known to reach lengths of 13 cm TL (BAMZ specimen 1998-172-005). Females are typically larger than males. Sex ratios among the Bermuda sub-populations, where they were not 1:1, always biased towards females (Outerbridge et al., 2007a). Selective predation may account for this, given the fact that males are more conspicuous during the breeding season.

Surveys undertaken between 2004 and 2017 have shown that abundance varies greatly between the different extant sub-populations, ranging from approximately 400 fish (Warwick Pond in 2005 and Riddell's Bay pond in 2013) to nearly 70,000 fish (Mangrove Lake in 2011) (Table 1). Killifish abundance can also vary greatly even within the same pond over time. For example, the sub-population in Mangrove Lake had an estimated 11,325 fish in it in 2004 (Outerbridge et al., 2007a) and 69,373 by 2011 (Copeland, 2013). Trott's Pond was reported to comprise approximately 8,000 individuals in 2004 (Outerbridge et al., 2007a), but by 2011 it had reduced to an estimated 1,900 individuals (Copeland, 2013). In past decades this sub-population was so reduced in number that it was believed to have disappeared completely (see Smith-Vaniz et al., 1999).

Table 1. Petersen population estimates for *Fundulus* on Bermuda

Species	**Location**	**2004-2005* (SE +/-)**	**2011-2012****	**2013*****
F. relictus	Lover's Lake	8,508 (1,347)	7,907	-
F. relictus	Bartram's Pond	1,793 (224)	1,244	-
F. relictus	Cooper's Island Pond	-	2,329	-
F. bermudae	Blue Hole Bird Pond	5,394 (480)	12,922	-
F. bermudae	West Walsingham	2,202 (178)	5,389	-
F. bermudae	Shelly Bay Pond	-	1,012	-
F. bermudae	Trott's Pond	7,926 (1,576)	1,934	-
F. bermudae	Mangrove Lake	11,325 (1,884)	69,373	-
F. bermudae ?	Warwick Pond	436 (13)	-	-
F. bermudae ?	Seymour's Pond	-	10,316	-
F. bermudae ?	Riddell's Bay #15 Pond	-	-	417

? Indicates a possible new *Fundulus* species
*Outerbridge, 2007a
**Copeland, 2013
***Outerbridge, unpublished data

Estimates of abundance for the sub-populations in the East Walsingham Ponds, the Sea Swept Farm Pond, Evan's Pond, and the Madagascar Pond at BAMZ are not available.

While it has been shown that abundance varies over time, sustainable levels of killifish (i.e. sub-populations >5,000) appears to be maintained in at least three ponds (Mangrove Lake, the Blue Hole Bird Pond, and Lover's Lake. It is also likely that the Seymour's Pond sub-population is sustainable, although additional surveys are needed to determine this. Furthermore, Trott's Pond and the West Walsingham Ponds appear to periodically have sustainable sub-populations. It would be desirable to understand what causes the observed fluctuations in fish abundance.

Reproduction

Outerbridge et al. (2007b) examined the reproductive periodicity of the *F. bermudae* population in Mangrove Lake. A distinct annual pattern was evident 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. It is unknown what the environmental cues are that elicit spawning in the Mangrove Lake population, however research into the reproductive biology of *F. heteroclitus* has linked spawning cues to environmental factors such as photoperiodicity, temperature, and tidal cycle (Hines et al., 1985; Taylor, 1986). 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 and Hata, 1984) and have been found carrying up to 108 ripe eggs at a single time (Outerbridge et al., 2007b). These eggs are large and sticky allowing them to easily adhere to solid surfaces.

Able and Hata (1984) described the spawning site preferences of *F. bermudae* and *F. relictus,* under controlled conditions. They found that spawning site selection varied between the species; female *F. bermudae* were reported to deposit nearly 75% of their eggs on a spawning mop provided by the researchers, while nearly 94% of the *F. relictus* eggs were deposited on the glass surface of the aquarium. Observations in the field have shown that female *F. bermudae* deposit eggs on the prop roots of red mangroves (Fig. 6) and on the leaf litter lying among the prop roots (M.O. pers. obs.).

Figure 6. Bermuda killifish eggs on the prop roots of red mangroves.

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 only one or two. Both authors also reported that the number of eggs a female is capable of producing is directly related to fish length.

Life Cycle

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 and Stiven, 1978). Numerous studies on *F. heteroclitus* have revealed that juveniles grow quickly after hatching, reaching sizes between 45-82 mm by the start of the following summer. Sexual maturity is reached within the first year and they do not appear to live longer than four years (Fritz and Garside, 1975; Valiela et al., 1977; Kneib and Stiven, 1978). The lifespan of Bermuda's killifishes is unknown, however individuals have lived for five years in captivity (J. Gray, pers. comm.).

E. Current Threats

Habitat Loss

The main reason for the decline in abundance and distribution of Bermuda's killifishes is habitat modification during the first half of the twentieth century. Historically, killifish were known from the brackish ditches at the back of Hamilton (Pembroke Marsh), the ditches around Devonshire Marsh, Paget East swamp (a possible reference to the mangrove swamp in Hungry Bay), Stocks Point (St. David's Island), and the muddy bays and mangrove swamps about St. George's (Hurdis, 1897; Boëtius and Boëtius, 1967; Smith-Vaniz et al., 1999). Since the island's colonization in 1609 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 totaling 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 (Sterrer and Wingate, 1981). It has been estimated that during the 1970's 100 tons of garbage was dumped daily into the Pembroke Marsh complex. 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 one third 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 and Wingate, 1981). This single act of environmental damage forever altered the water quality and marine communities within the Castle Harbour and St. George Harbour area, the latter having been mentioned as an area frequently containing killifish (Drummond-Hay unpublished notes in Smith-Vaniz et al., 1999).

Pollution

Although not industrialised, Bermuda is characterised by high levels of localised anthropogenic pollution (Jones, 2011). Recent investigations of the health status of the pond environment on Bermuda suggest that there is a suite of contaminants of concern that are having detrimental effects on the resident fauna. Tissue residue analyses from a range of taxa, including cane toads *Rhinella marinus*, mosquitofish *Gambusia holbrooki*, killifish *Fundulus bermudae*, and red-eared sliders *Trachemys scripta elegans* collected from a variety of contaminated wetlands across Bermuda have shown that petroleum hydrocarbons, polycyclic aromatic hydrocarbons and trace metals are being accumulated and induce developmental malformations, endocrine disruption, liver and gonad abnormalities plus immunological stress (Fort et al., 2006; Fort et al., 2006; Bacon, 2010; Bacon et al., 2012). Entry of contaminants into the wetlands comes through storm-water run-off from adjacent roadways, aerial deposition, and leachate from ground-water sources (Fort et al., 2006). Some killifish populations living in urban estuaries along the eastern seaboard of the USA have been found to possess the ability to adapt to toxic pollution (i.e. dioxins, heavy metals and hydrocarbons) (Whitehead et al., 2017); however decreased reproductive fecundity has been observed under controlled conditions (Fort et al., 2015) and offspring may be more susceptible to other stressors (Meyer et al., 2003). This suggests that Bermuda's killifishes may be able to persist in polluted ponds but at the cost of compromised reproduction.

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 gray snappers *Lutjanus griseus* (Outerbridge, 2006) and various herons (Wingate 1994; M.O. pers. obs.). The mosquito fish *Gambusia holbrooki* has been reported to be predatory towards a wide variety of fish species around the planet (primarily towards eggs and fry) and have caused, or contributed to, the elimination of many populations of fishes with similar ecological requirements (Meffe, 1985; Page and Burr, 1991). This species was deliberately introduced in 1928 to help control mosquitoes and is prevalent in many wetland habitats throughout Bermuda, including Bartram's Pond, the east Walsingham Ponds, Trott's Pond, Mangrove Lake, Warwick Pond, and the Sea Swept Farm Pond (M.O. pers. obs.).

Collection for aquaria

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.

F. Current Conservation Actions

Translocations

Between 1976 and 2012 over 3,700 killifishes of known identity were intentionally translocated from their original ponds to 13 man-made ponds as a precaution against possible extinction events. Table 2 summarizes these transfer histories. Success (indicated by the presence of adults and evidence of juvenile recruitment three years after the original introduction) was documented in all but two ponds (David's Pond in Paget Marsh and the water hazard on the 17th hole of the Port Royal golf course). The reason for the two failures are unclear. Another pond (the fresh water pond on Nonsuch Island) had a thriving sub-population of killifish for decades but the pond has slowly turned into a fresh water marsh with almost no open water and the killifish seem to have disappeared (Outerbridge, 2006). Furthermore, two additional ponds (the salt water pond on Nonsuch Island and the Wind Reach Pond) were unintentionally destroyed in the years following the killifish introductions, but the sub-populations were extant at the time of the losses. However, some past translocation efforts have proven very successful. For example, Seymour's Pond had 400 fish introduced into it in May 2011. One year later this sub-population had increased to an estimated 10,316 individuals (Copeland, 2013).

Table 2. Summary of the killifish transfer histories among the man-made ponds of Bermuda

Pond name	Species	Year of translocation	Founding source	Number translocated	Status in 2017*
Nonsuch Island (fresh)	*F. bermudae*	1976	Trott's Pond	n/a	extirpated
Nonsuch Island (salt)	*F. bermudae*	1993	Mangrove Lake	53[a]	extirpated
Bartram's Pond	*F. relictus*	1986 & 2008	Lover's Lake	n/a & 766[b]	extant
Blue Hole Bird Pond	*F. bermudae*	1995 & 2008	West Walsingham	50[c] & 420[b]	extant
Cooper's Island Pond	*F. relictus*	2008 & 2009	Lover's Lake	400 & 334[b]	extant
Wind Reach Pond	*F. bermudae?*	2008	Warwick	20[b]	extirpated
Sea Swept Farm Pond	*F. bermudae?*	2008	Evan's Pond	103[b]	extant
Shelly Bay Pond	*F. bermudae*	2009	West Walsingham	719[b]	extant
David's Pond	*F. bermudae*	2009	Wind Reach	118[b]	extirpated
Port Royal (#17)	*F. bermudae?*	2010	Wind Reach	200[b]	extirpated
Seymour's Pond	*F. bermudae?*	2011	Wind Reach	400[b]	extant
Riddell's Bay (#15)	*F. bermudae?*	2012	Evan's Pond	100[b]	extant
BAMZ (Madagascar)	*F. bermudae?*	2012	Wind Reach	35[b]	extant

*confirmed by author or Wolf (2017)
n/a figures not available
[a] Cotter (1993)
[b] M. Outerbridge, unpublished data
[c] J. Madeiros, personal communication

Resolution of genetic identity

It has been suggested that Bermuda's killifish populations should be separated into three different management groups;

- Eastern group comprising the *F. relictus* populations in Lover's Lake, Bartram's Pond, and Cooper's Island,
- Central group comprising the *F. bermudae* populations in the various Walsingham Ponds, the Blue Hole Bird Pond, Shelly Bay Pond, Trott's Pond, and Mangrove Lake,
- Western group made up of the *F. bermudae* populations in Warwick Pond, Evan's Pond, Seymour's Pond, the Sea Swept Farm pond, and the pond on the Riddell's Bay golf course.

In order to preserve the genetic differences between these different groups, very effort should be made to not mix them during future translocations.

Wetland Restoration

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 introduce *Fundulus*. At the time of writing, the excavation of Eve's Pond in Hamilton Parish had just been completed. Plans are currently being made to stock the pond with killifish.

Prohibition on importing killifishes in the *Fundulus heteroclitus-grandis* complex

All animals (including fish) arriving into Bermuda must be accompanied by an import permit together with an original health certificate; however in 2018 the Department of Environment and Natural Resources created a policy to manage the importation of potentially invasive non-native (i.e. alien) animal species. The objective of this policy was to prevent the introduction of species that are deemed to have a negative impact on human or domestic/captive animal health as well as the ecology and economy of Bermuda. Killifishes from the *F. heteroclitus-grandis* complex are included on the prohibition list because they are closely related *F. bermudae* and *F. relictus* and are likely to hybridize with theses island endemics should they find their way into Bermuda's wetlands.

PART II: RECOVERY

A. Recovery goal

The principal aim of this Recovery Plan is to increase abundance 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 anthropogenic sources.

The short term goal (5 years) is to continue to research the biology and ecology of Bermuda's killifishes, identify ponds that are suitable for killifish translocation, and rank them according to suitability.

The long term goal (30 years) is to increase island-wide and range of Bermuda's killifishes, remediate existing wetland habitats, and create additional ponds for killifish habitation.

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 species, or at least the degree of intra-population genetic sub-structuring, is known).
- An updated assessment has been undertaken for all extant sub-populations.
- All potential habitats suitable for growth, reproduction and survival are identified, assessed, and restored.
- Each species of *Fundulus* inhabits at least three separate ponds.
- Each species inhabits at least one pond that has been officially designated as 'Critical Habitat'.
- Long-term, sustainable levels of killifish are reached in each pond that has been designated as 'Critical Habitat' (i.e. population >5,000).

These overall objectives translate into specific targets outlined below:

<u>Short-term target (5 years).</u> To ensure that by 2025 all studies necessary for development of effective management will be complete, and that some ponds will be identified as "Critical Habitat" as defined under the Protected Species Act (2003). This short-term goal includes re-surveying the extant populations, with a particular focus on Evan's Pond and Warwick Pond. During this time, the identification and assessment of "health" status of current and potential habitats will be conducted.

<u>Long-term target (30 years).</u> The restoration of Bermuda's wetlands, including the remediation of existing ponds and excavation to create new ponds, will lead to an increase in both the area of occupancy and abundance for both species. Aquaculture should be investigated as a tool to assist with the increase of species abundance, especially if donor populations are not large enough to translocate from directly.

C. Recovery strategy

The strategy for recovery revolves around assessing the "health" status (namely water and sediment quality) of Bermuda's ponds, their remediation, and in the active intervention required for increasing the species distribution to a greater range. The selection of ponds for translocation is critical as habitat quality appears poor in several locations, based on previous sediment and tissue analyses (J. Bacon, pers. comm.). This further drives the need for wetland remediation activities and controlling as much as possible contamination input from external sources. Only after suitable locations have been identified can the transferal of fish occur, either through the direct collection and immediate transfer of individuals (if the founding population is deemed to be large enough) or through collection and propagation via aquaculture. The multi-species fish hatchery on the Coney Island field station could be used for this purpose.

Translocation of killifish of known species to new ponds is the only way to ensure range increase. Because of past translocation efforts, the eastern group management unit (i.e. *F. relictus*) now resides in three ponds, the central group management unit (*F. bermudae 1*) resides in six ponds, and the western group management unit (*F. bermudae 2*) also resides in six ponds.

At present, translocations should be kept tightly bound to geography. To prevent hybridization between *Fundulus relictus* and *Fundulus bermudae*, and mixing of genetic stock with the third suspected species believed to inhabit the western ponds of Bermuda, killifish should never be released into a pond that already contains an extant sub-population unless it is being done as part of a planned conservation programme using fish of known identity (i.e. the donor fish come from the original founding population) and the fish are disease free. Additionally, ponds that contain killifish whose donor source is not known, or if the fish are suspected to have come from different donor populations, should not be used in future translocation efforts.

Potential future translocations (Fig. 7) to be considered are as follows:

- *F. relictus* from Lover's Lake to the main pond on Coney Island,
- *F. bermudae* from Trott's Pond to Eve's Pond,
- *F. bermudae* from Mangrove Lake to the main pond in Devonshire Marsh (at the end of Marsh Lane) as well as to the main pond in the Edmund Gibbon's Nature Reserve,
- *F. bermudae* from Evan's Pond to both ponds in the Somerset Long Bay nature reserves as well as to the lagoon on Ireland Island.

Periodic monitoring of the newly established sub-populations will be required in order to determine short-term and longer-term survival.

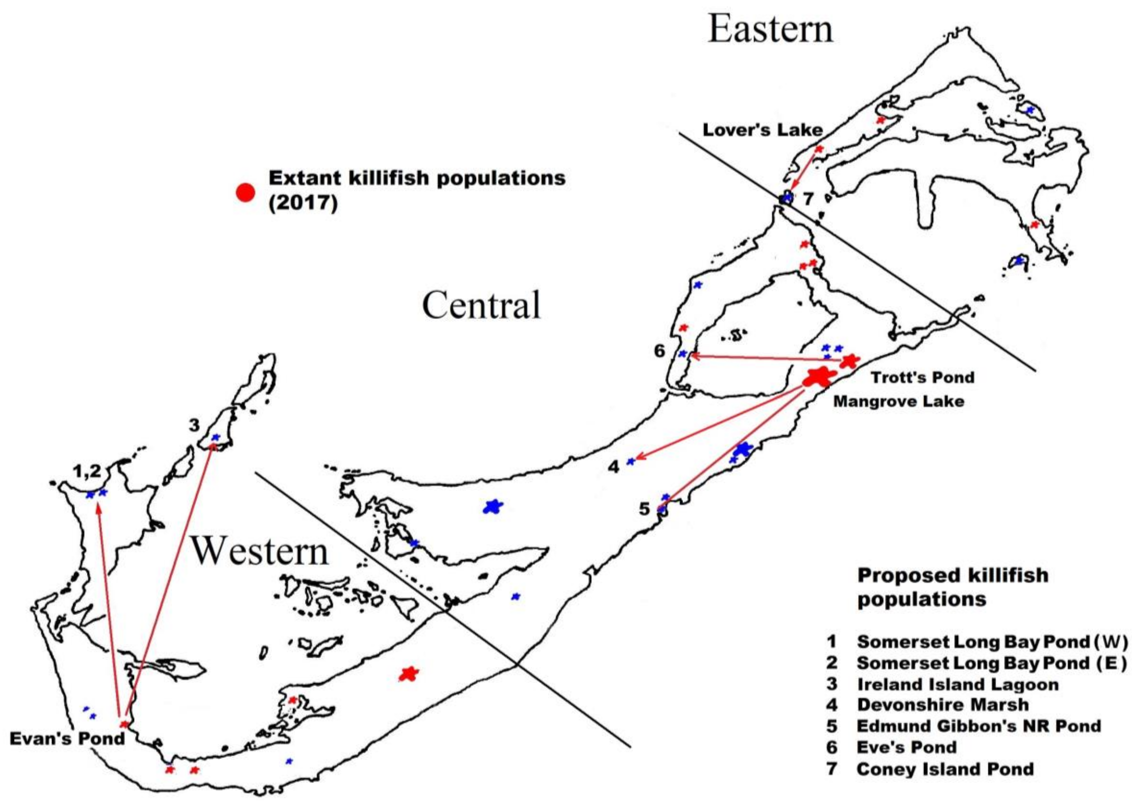

Figure 7. Schematic diagram of proposed future killifish translocations within the three different management units

D. Tools available for strategy

Population assessments

Estimates of abundance are calculated through capture-mark-recapture surveys. Capture is accomplished using baited minnow traps, provided they are not left for too long in the ponds (which greatly increases the chances of causing death through asphyxiation). Marking captured fish can be accomplished using various methodologies to create identifiable features, however these studies require that fish are released in good condition and the tags or marks must not compromise the behavior and survival of the fish (see reviews in Thorsteinsson, 2002; Pine et al., 2003). Visible implant elastomer (VIE) is one product that has been used with effectiveness on Bermuda's killifishes (Outerbridge et al., 2007a) but it is time consuming and labour intensive. Other marking and tagging methodologies that may prove effective include fin clipping (Velázquez-Velázquez and Schmitter-Soto, 2004) or the application of thread tags (Solomon-Lane and Hofmann, 2018).

Wetland remediation

Examples of wetland remediation include phytoremediation, in which plants are used to extract persistent contaminants from surrounding substrate, as well as employing various chemical and biological remediation techniques. Chemical remediation methods include reducing or eliminating inputs of contaminants from point sources, natural sediment remediation by biodegradation and chemical degradation, and active sediment remediation by removal or by in situ treatment; biological remediation methods include enhancing populations of target organisms (see reviews in Wilcox and Whillans, 1999). Some wetland plants have been shown to sequester petroleum hydrocarbons (Lin and Mendelssohn, 1998), polycyclic aromatic hydrocarbons (Lin and Mendelssohn, 2009) and trace metals (Weis and Weis, 2004) from wetland sediment and store them below ground in roots or concentrate them in aerial tissues (e.g. leaves and stems). Depositing clean sediment (e.g. diatomaceous earth) over contaminated sediment is yet another technique of wetland remediation that can diminish the risk of biological contact, however it should not be considered without first assessing its impact on the water column and aquatic biota of the ponds. Additionally, the creation of buffer zones (e.g. road-side reed beds) between road drains and freshwater wetlands would help to reduce direct in-put of pollutants by serving as a filter for contaminants entering as road runoff (see Revitt et al., 1997; Cooper, 1999). Introduction of oxygenated air into the organic sediment of contaminated areas promotes natural biological degradation of some contaminants (e.g. polycyclic aromatic hydrocarbons) by increasing the activity of indigenous bacteria that are capable of metabolizing pollutants (D. Fort, pers. comm.). This has been recently trialed in some of Bermuda's wetlands and appears to significantly reduce the level of polycyclic aromatic hydrocarbons after one year (J. Bacon, pers. comm.).

Aquaculture
Green et al., (2010) and Ramee et al., (2019) provide excellent accounts of the aquaculture of *F. grandis* which should be applicable to the culture of *F. bermudae* and *F. relictus*.

E. Step-down narrative of work plan

The actions needed to achieve recovery are as follows:

1. Resolve the phylogenetic relationships between the existing sub-populations.
2. Update the population assessments.
3. Identify and restore wetland habitats suitable for killifish introduction.
4. Increase abundance and expand area of occupancy through translocation and aquaculture (if deemed necessary).
5. Designate Lover's Lake as "Critical Habitat' for *Fundulus relictus* and Mangrove Lake and Seymour's Pond as "Critical Habitat' for *Fundulus bermudae* as described under the Protected Species Act.

1. Resolve the phylogenetic relationships between the existing sub-populations.

   Actions:
   - Collect fresh tissue samples (if required),
   - Analyze samples,
   - Produce guidelines for future management activities,
   - Add new *Fundulus* species on Protected Species Order (if appropriate).

   Work Team: Department of Environment and Natural Resources and collaborative institution undertaking genetic analyses

Team Leader: Genetics laboratory
Assistance: Graduate student or laboratory technician
Outputs: Determination of taxonomic diversity between extant populations in Bermuda, graduate thesis and/or peer reviewed publication, amended legislation for protection of new species

2. Update the population assessments.

   Actions:
   - Confirm species presence in the ponds (presence/absence survey),
   - Undertake capture-mark-recapture surveys on extant sub-populations,
   - Compare results with past surveys.

   Work Team: Department of Environment and Natural Resources and student or volunteers
   Team Leader: Department of Environment and Natural Resources
   Outputs: Report on the current demographic characteristics of Bermuda's killifishes (i.e. estimates of abundance, sex ratios, and different size classes)
   List of equipment required: Truck, row boat, minnow traps, bait, anesthetic (e.g. clove oil), tagging equipment, life support system for fish (e.g. buckets, SCUBA tank, air stones, dip nets, etc.)

3. Identify and restore wetland habitats suitable for killifish introduction.

   Actions:
   - Finish studying the physical characteristics of the ponds listed on Table 3,
   - Perform sediment and water quality analyses of ponds,
   - Identify suitable remediation activities and commence,
   - Control of invasive species.

   Work Team: Department of Environment and Natural Resources, Bermuda Zoological Society, and Fort Environmental Laboratories (USA), land owners of ponds identified for remediation
   Team Leader: Department of Environment and Natural Resources
   Assistance: Financial donors
   Outputs: A database of toxic burden for each pond, prioritized list of sites identified as being suitable for killifish survival.

4. Increase abundance and expand area of occupancy through translocation and aquaculture (if deemed necessary).

   Actions:
   - Introduce large numbers (minimum 500) of killifish in equal sex ratio of known identity to suitable ponds,
   - Monitor the introduced sub-population(s) by capture-mark-recapture method,

- Carry out additional releases once the initial translocation is proven successful to ensure sustainability of population and optimized genetic diversity,
- Collection of brood stock (if necessary),
- Egg collection and incubation (if necessary),
- Larval rearing and grow-out of juveniles (if necessary),
- Offspring used for translocation programme (if necessary).

Work Team: Department of Environment and Natural Resources, land owners of ponds which will be stocked with killifish
Team Leader: Department of Environment and Natural Resources
Assistance: Volunteers for field work, staff to manage aquaculture
Outputs: Assessment of killifish sub-populations following translocations, overall increase in species abundance and range, report on culture techniques for Bermuda killifish
List of equipment required: Truck, row boat, minnow traps, bait, life support system for fish (e.g. buckets, SCUBA tank, air stones, dip nets, etc.); aquaculture equipment (see Green et al., 2010; Ramee et al., 2019)

5. Designate Lover's Lake as "Critical Habitat' for *Fundulus relictus* and Mangrove Lake and Seymour's Pond as "Critical Habitat' for *Fundulus bermudae* as described under the Protected Species Act.

   Actions:
   - Create maps of above locations showing boundaries,
   - Publish notice in the official gazette for public inspection,
   - Create new order.

   Work Team: Department of Environment and Natural Resources, land owners of ponds
   Team Leader: Department of Environment and Natural Resources
   Assistance: Attorney General's Chambers
   Outputs: Amended legislation for protection of habitat

F. Estimated Date of down listing

The population assessments can be accomplished within a six month time period but it is anticipated that it will take up to five years to identify and restore key habitats, and three years to verify the success of the proposed translocations. It is only once these are attained that down listing (or removal) of all species will be considered. Re-assessment of all species should be done every 10 years.

PART III: IMPLEMENTATION

<u>Priority 1</u>: An action that must be taken to prevent extinction or to prevent the species from declining irreversibly.
<u>Priority 2</u>: 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.
<u>Priority 3</u>: All other action necessary to provide for full recovery of the species.

Priority #	Task #	Task description	Task Duration	Responsible Party
1		**Translocation and/or aquaculture**		
	1	Series of direct translocations and monitoring	6 months	DENR
	2	Brood stock collection	1 month	DENR
	3	Egg collection & incubation	6 months	DENR
	4	Larval rearing & juvenile grow-out	6 months	
	5	Translocation of juveniles to ponds	36 months	DENR
2		**Update population assessments**		
	6	Presence/absence surveys	1 week	DENR
	7	Undertake capture-mark-recapture	6 months	DENR
	8	Compare with previous surveys	1 day	DENR
3		**Identify and restore ponds**		
	9	Complete studying the physical characteristics	12 months	DENR
	10	Sediment and water quality analyses	12 months	DENR, BZS
	11	Remediation	ongoing	DENR, BZS
	12	Control of invasive species	ongoing	DENR
4		**Genetic investigation**		
	13	Collect tissue samples	1 week	DENR
	14	Analyze samples	3 months	Partner lab
	15	Produce management guidelines	1 day	DENR
	16	Protection of all species identified	6 months	DENR
5		**Designate 'Critical Habitats'**		
	17	Create maps	1 day	DENR
	18	Gazette notice	1 month	DENR
	19	Create order	6 months	AG chambers

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APPENDIX

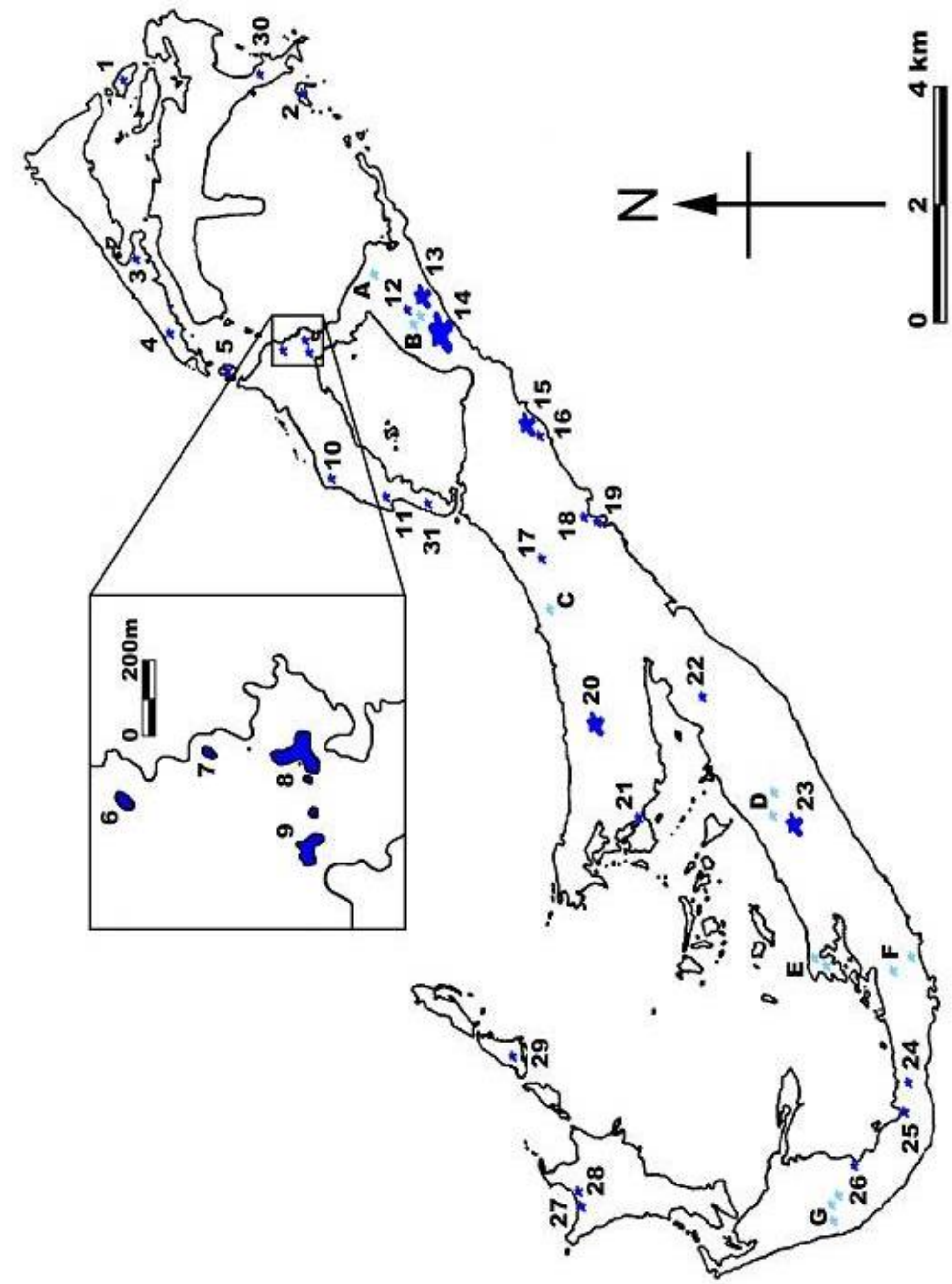

Figure 8. Map showing the locations of various ponds across Bermuda

Map Legend for Fig. 8:

1. Paget Island Lagoon
2. Nonsuch Island Pond
3. Bartram's Pond
4. Lover's Lake
5. Coney Island Pond
6. Blue Hole Bird Pond
7. Blue Hole Grotto
8. East Walsingham Ponds
9. West Walsingham Ponds
10. Davis Pond (aka the Crawl Pond)
11. Shelly Bay Pond
12. Compston's Pond
13. Trott's Pond
14. Mangrove Lake
15. Spittal Pond
16. Heron & Round Ponds
17. Devonshire Marsh Pond
18. Cloverdale Pond
19. Gibbon's Nature Reserve Pond
20. East Pembroke Marsh Pond
21. Fairyland's bridge pond
22. David's Pond (Paget Marsh)
23. Warwick Pond
24. Seymour's Pond
25. Sea Swept Farm Pond
26. Evan's Pond
27. Somerset Long Bay Pond (west)
28. Somerset Long Bay Pond (east), formerly known as Pitman's Pond
29. Ireland Island Lagoon
30. Cooper's Island Pond
31. Eve's Pond

A. Tucker's Point golf course (2 ponds)
B. Mid Ocean golf course (4 ponds)
C. Ocean View golf course (1 pond)
D. Belmont golf course (2 ponds)
E. Riddell's Bay golf course (3 ponds)
F. Southampton Princess golf course (2 ponds)
G. Port Royal golf course (4 ponds)

Table 3. Summary of physical properties important to killifish survival for 47 ponds across Bermuda

Pond Name	Salinity*	Temp. °C range (mean)	Area of open water (acres)	Pollution profile available?	Road drain(s) present?	Ownership
†Bartram's Pond	M	13.9-31.7 (23.3)	0.56	N	N	BDA Audubon Soc.
Φ Lover's Lake	M	18.0-28.5 (23.7)	1.01	Y	N	BDA Government
†Paget Island lagoon	M	unknown	0.46	N	N	BDA Government
†Coney Island	M	unknown	0.97	N	Y	BDA Government
†Nonsuch Island Pond	FW	unknown	0.05	N	N	BDA Government
†Blue Hole Bird Pond	M	16.2-28.3 (23.2)	0.22	N	N	BDA Government
Φ Blue Hole Grotto	M	unknown	0.08	N	N	BDA Government
Φ West Walsingham Ponds	M	12.4-37.9 (23.7)	0.87	N	N	Wilkinson Trust
Φ East Walsingham Pond	M	19.0-27.7 (23.2)	1.95	N	N	Walsingham Trust
†Tucker's Point Club #6 pond	FW	unknown	1.26	N	N	Private
†Compston Pond	BR	unknown	0.60	N	N	Private
†South Pond	BR	11.6-39.0 (23.8)	0.44	Y	N	Private
†North Pond	BR	unknown	0.99	N	N	Private
Φ Trott's Pond	BR	16.0-31.0 (24.5)	2.88	N	Y	Private
Φ Mangrove Lake	M	13.7-33.6 (24.6)	9.89	Y	Y	Private
Φ Davis Pond (aka the Crawl Pond)	M	unknown	0.47	N	Y	Private
†Shelly Bay Marsh Pond	M	unknown	0.10	N	N	BDA Government
Φ Spittal Pond	BR	16.0-37.5 (32.0)	7.42	N	N	BDA National Trust
†Round Pond	BR	unknown	0.25	N	N	BDA National Trust
†Heron Pond	BR	unknown	0.18	N	N	BDA National Trust
†Cloverdale Pond	FW	17.0-32.0	0.35	Y	Y	Private
Gibbons Nature Reserve Pond	FW	unknown	0.20	N	N	BDA Audubon Soc.
†Ocean View Club #9 pond	FW	unknown	0.12	Y	N	BDA Government
†Devonshire Marsh Pond	FW	unknown	0.22	N	N	Private
†East Pembroke Marsh Pond	FW	unknown	4.07	N	Y	BDA Government
†Fairyland's Bridge Pond	M	unknown	1.75	N	N	Private
†David's Pond (Paget Marsh)	FW	13.6-31.6 (17.7)	0.20	Y	Y	BDA Audubon Soc.
Warwick Pond	BR	10.8-39.6 (24.0)	2.76	Y	Y	BDA National Trust
†Belmont Hills Club #6/7 pond	FW	unknown	0.77	N	N	Private
†Belmont Hills Club #13 pond	FW	unknown	0.50	N	N	Private
†Riddell's Bay Club #7 pond	F?	unknown	0.22	N	N	Private
†Riddell's Bay Club	M	unknown	0.03	N	N	Private
†Riddell's Bay Club #15 pond	M	unknown	0.31	N	N	Private
†Southampton Princess #3 pond	FW	unknown	0.94	N	N	Private
†Southampton Princess #17 pond	FW	unknown	0.17	N	N	Private
†Seymour's Pond	BR	18.7-30.9	0.79	Y	Y	BDA Audubon Soc.
†Sea Swept Farm Pond	BR	unknown	0.45	N	Y	Private
†Port Royal #1 pond	FW	unknown	0.29	N	N	BDA Government
†Port Royal #2 pond	FW	unknown	0.96	N	N	BDA Government
†Port Royal #3 pond	FW	unknown	0.42	N	N	BDA Government
†Port Royal #17/18 pond	FW	unknown	1.35	N	N	BDA Government
Φ Evan's Pond	M	17.0-32.0 (24.6)	1.79	Y	Y	Private
†Somerset Long Bay Pond (west)	FW	unknown	0.51	N	N	BDA Audubon Soc.
†Somerset Long Bay Pond (east)	FW	19.0-33.0	0.72	Y	N	Buy Back BDA
†Ireland Island Lagoon	M	unknown	3.97	N	N	BDA Government
†Cooper's Island Pond	M	unknown	0.24	N	N	BDA Government
†Eve's Pond	BR	unknown	unknown	N	N	Buy Back BDA

† ponds that have been significantly modified by humans (often entirely man-made); Φ natural ponds
*FW = freshwater <2 psu; BR = brackish water 2-29 psu; M = marine water >30 psu

Lover's Lake

Lover's Lake is a one acre marine pond situated in a government-owned nature reserve in St. George's Parish (Fig. 9) and is inhabited by *Fundulus relictus*. The pond is located within a limestone basin and is completely fringed by a dense growth of black mangrove trees *Avicennia germinans*. It 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. Appreciable amounts of sea water enters through this opening resulting in a flushing rate of ca. 60% and a tidal range of 51.5 cm. The mean depth is 91 cm, although the maximum was recorded to be 441 cm (Thomas et al., 1991) (Fig. 10). Average annual surface water temperatures ranged from 18-28.5°C (mean 23.7°C) and salinities varied from 22-37 psu (mean 30.3 psu) (Thomas et al., 1991).

Figure 9. 2012 aerial photograph of Lover's Lake

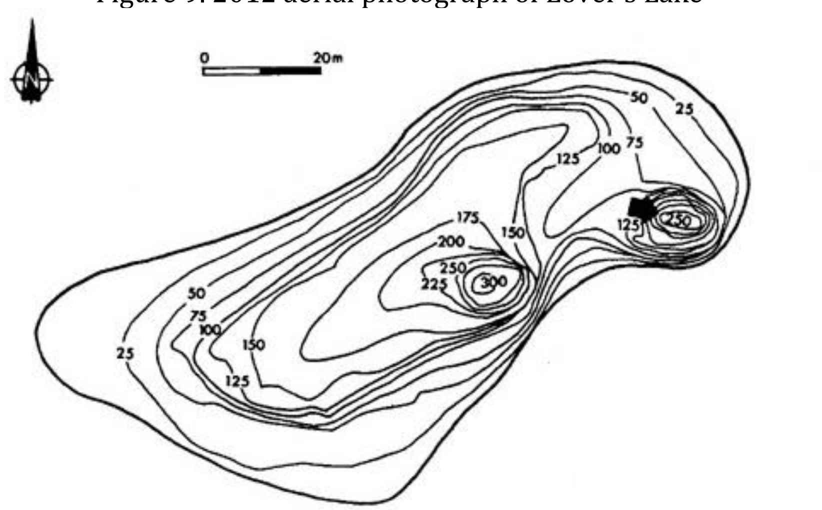

Figure 10. Bathymetry map for Lover's Lake
(Depth is in cm. Black arrow indicates area of tidal exchange)
Figure adapted from Thomas et al., 1991

Bartram's Pond

Bartram's Pond (Fig. 11) was originally about 0.75 acres in size and bordered by mangroves, prior to being filled with garbage by the middle of the 20th century. Dredging began in 1983 which resulted in the creation of a half-acre marine pond containing two small islets. The new pond is situated in a 2.4 acre nature reserve in St. George's Parish. The mosquito fish *Gambusia holbrooki* was introduced in 1985. *Fundulus 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. This pond is connected to Mullet Bay by a tidal channel which runs under the road. The mean depth is 123 cm and the depth range was 32-178 cm. Average annual surface water temperatures is known to range from 13.9-31.7°C (mean 23.3 °C) and salinities can vary from 27-37 psu (mean 31 psu) (Outerbridge and Thomas, unpublished data).

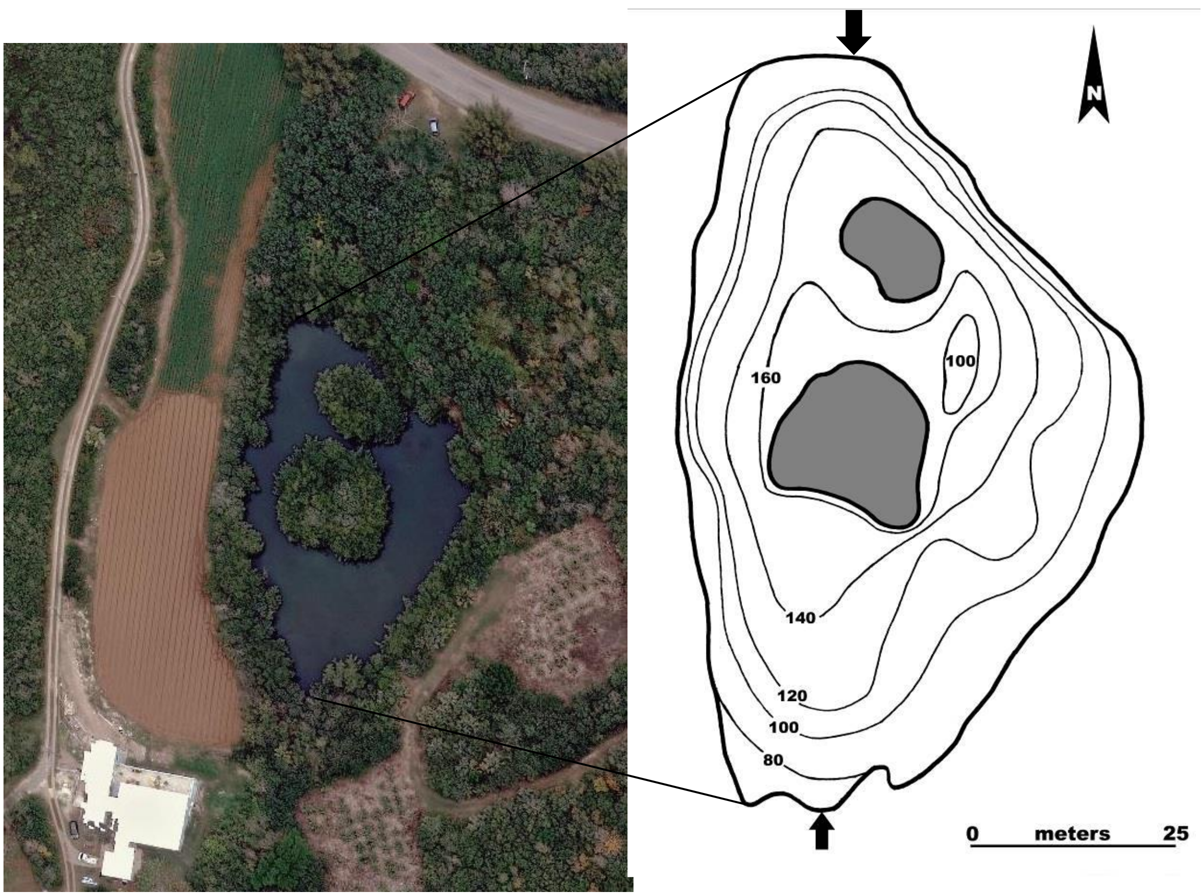

Figure 11. 2012 aerial photograph of Bartram's Pond (left) and map of bathymetry (right) (Depth is in cm. Black arrows indicate areas of tidal exchange)

Cooper's Island Pond

This 0.24 acre tidal, marine pond (Fig. 12) was created in 2007 and is accessible via a wood boardwalk. *Fundulus relictus* (n=400) were introduced from Lover's Lake in 2008 and a further 334 fish were added in 2009 after the original translocation proved successful. A survey in 2011 revealed that the sub-population had increased to an estimated 2,329 fish (Copeland, 2013). A small salt marsh exists to the west of the main boardwalk which is used by the killifish during periods of high tide. This boardwalk provides the best opportunity to view Bermuda's killifish in the wild. Red and black mangroves were deliberately not planted at this location in order to keep the salt marsh intact. The underwater topology, as well as the annual variation in water temperature and salinity, for this pond has not been studied.

Figure 12. 2012 aerial photograph of the Cooper's Island Pond

Blue Hole Bird Pond

The original pond was filled during the 1920s and 1930s; however in 1994 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 0.22 acre pond (Figs. 13 and 14), located on a government-owned nature reserve and park in Hamilton Parish, is marine (ca. 34 psu), tidal and supports a sub-population of *F. bermudae* deliberately introduced by Parks Department personnel from one of the West Walsingham Ponds. Red mangrove trees are found growing around the perimeter of the pond. The mean depth of this pond is 66 cm and the depth range is 38-99 cm (Outerbridge and Thomas, unpublished data). The annual variation in water temperature and salinity is unknown.

Figure 13. 2012 aerial photograph of the Blue Hole Bird Pond

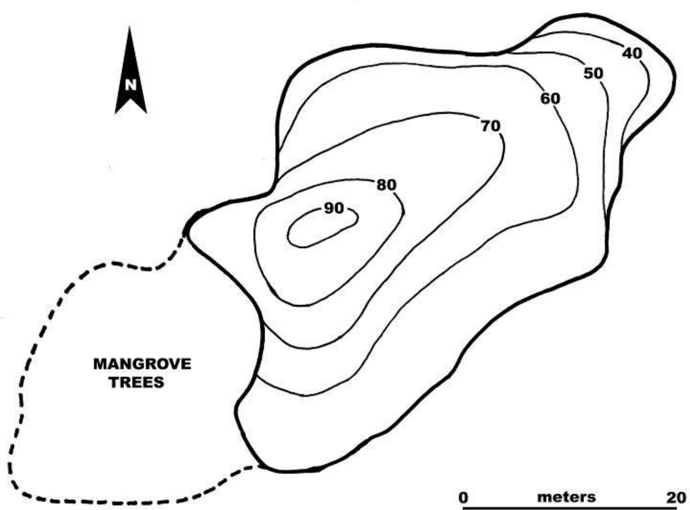

Figure 14. Bathymetry map for the Blue Hole Bird Pond
(Depth is in cm)

East Walsingham Pond

The 1.95 acre East Walsingham Pond, also known as the Walsingham Lagoon, is situated in a large tract of land called the Walsingham Nature Reserve in Hamilton Parish and is managed by a private Trust (Figs. 15 and 16). The pond originated from the collapse of a large cavern in the Walsingham Formation which subsequently filled with sea water. It is presently fringed by red mangrove trees, has vertical cliffs that descend five meters into the water in some areas, and has two distinct basins separated by a shallow sill of rock. The East Walsingham Pond is the deepest of all the ponds in Bermuda (618 cm) and averages 392 cm (Thomas et al., 1991). Oceanic connections are large, numerous and located close to the water surface thereby resulting in a comparatively large tidal range of 38.5 cm (Thomas and Logan, 1992). This pond is well known for its high and varied biodiversity of marine fishes, algae and sponges that colonize the exposed rock faces and red mangrove prop roots which surround the pond. *Fundulus bermudae* inhabit this pond but have proven very difficult to trap thereby preventing proper population assessments. Average annual surface water temperatures ranged from 19-27.7°C (mean 23.2°C) and salinity varied from 33-39.5 psu (mean 36.2 psu) (Thomas et al., 1991).

West Walsingham Ponds

The three bodies of water which comprise the West Walsingham Ponds (Fig.15) are also situated in the Walsingham Nature Reserve but are managed by a different private Trust. 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 two largest ponds. The largest of the ponds has at least three surface connections to the sea, accounting for the high tidal range observed in this pond. Widgeon grass *Ruppia maritima* is present and a number of marine fishes inhabit the ponds in addition to *F. bermudae,* including gray snappers *Lutjanus griseus*, mullets *Mugil liza*, pin fish *Lagodon rhomboids* and crested gobies *Lophogobius cyprinoides,* which suggests that salinity is close to that of sea water. The grey snappers in the main pond have been observed preying on the killifish (M.O. pers. obs.). There is also a salt marsh in the basin of the two main ponds which is heavily utilized by the killifish during high tides. The depths for the two largest ponds range from 15-280 cm (mean 97.3 cm). Water temperatures ranged from 12.4-37.9°C (mean 23.7°C) (Outerbridge and Thomas, unpublished data).

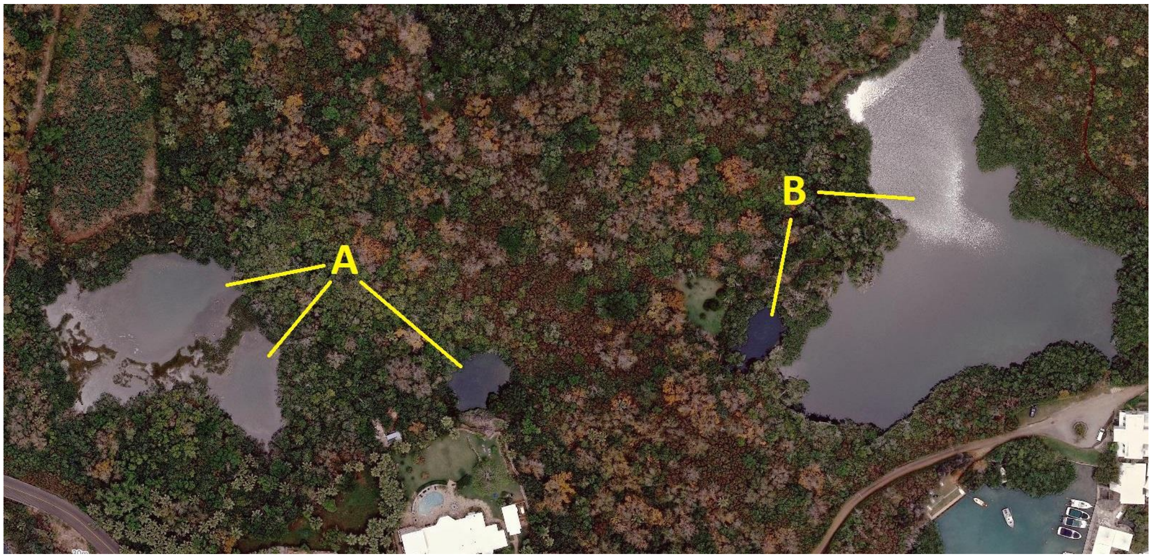

Figure 15. 2012 aerial photograph of the West (A) and East (B) Walsingham Ponds

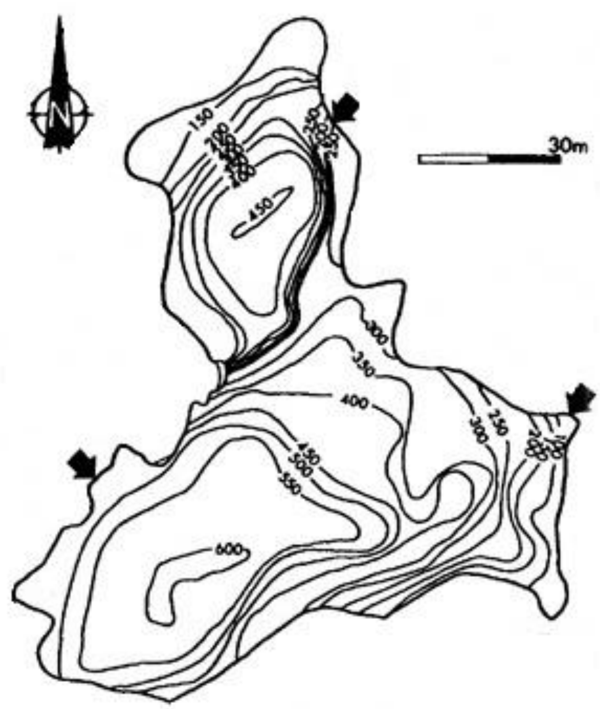

Figure 16. Bathymetry map for the main east Walsingham Pond
(Depth is in cm. Black arrows indicate areas of tidal exchange)
Figure adapted from Thomas et al., 1991

Mangrove Lake

Mangrove Lake (Fig. 17) is situated immediately to the west of Trott's Pond in Hamilton Parish. It is the largest pond on Bermuda (9.89 acres) and is partly bordered by a nature reserve, partly by a golf course, and partly by privately owned residential properties. It is believed to 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 (mean 134 cm), fairly even contours and a gently sloping shoreline. 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). Mangrove Lake contains the largest sub-population of *Fundulus bermudae* on Bermuda (Outerbridge, 2007a; Copeland, 2013) as well as a native population of diamondback terrapins *Malaclemys terrapin centrata* (Outerbridge, 2014). Average annual surface water temperatures are known to range from 13.7-33.6°C (mean 24.6°C) and salinity from 22-36 psu (mean 30.6 psu) (Thomas et al., 1991; Outerbridge, 2014).

Figure 17. 2012 aerial photograph of Mangrove Lake

Trott's Pond

Trott's Pond is situated on a privately owned golf course in Hamilton Parish (Fig. 18). It is currently 2.88 acres and originally formed between low Pleistocene sand dunes which were inundated by postglacial seas. Trott's Pond is a simple basin fringed by red mangrove trees and characterized by fairly shallow depths (mean 269 cm), with the deepest part (320 cm) at its center (Fig. 19) (Thomas et al., 1991). The connection to the ocean is small, located at the surface, and gives Trott's Pond a very low flushing rate of 0.5% and a small tidal range of 1.5 cm. Average annual surface water temperatures are known to range from 16-31°C (mean 24.5°C) and salinity from 24-34 psu (mean 29 psu) (Thomas et al., 1991; Outerbridge, 2014). *Fundulus bermudae* inhabit the pond along with a native population of diamondback terrapins *Malaclemys terrapin centrata* (Outerbridge, 2014).

Figure 18. 2012 aerial photograph of Trott's Pond

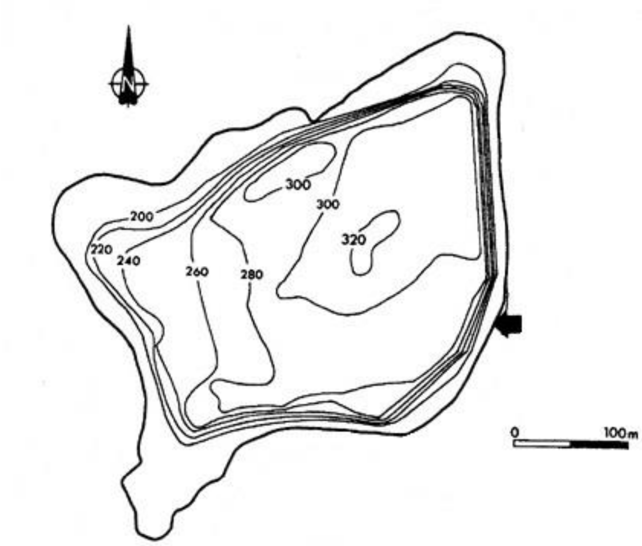

Figure 19. Bathymetry map of Trott's Pond
(Depth is in cm. Black arrow indicates area of tidal exchange)
Figure adapted from Thomas et al., 1991

Shelly Bay Pond

This nature reserve contains a relatively large red mangrove swamp (ca. 3 acres) with a shallow, 0.1 acre marine pond situated within it (Fig. 20). In 2009, 719 *Fundulus bermudae* from the West Walsingham ponds were introduced to this pond. The sub-population had increased to an estimated 1,012 individuals by 2011 (Copeland, 2013). The underwater topology, as well as the annual variation in water temperature and salinity, for this pond has not been studied.

Figure 20. 2012 aerial photograph of the Shelly Bay Pond

Warwick Pond

Warwick Pond (Fig. 21) is 2.76 acres in area and is one of the few naturally occurring seasonal freshwater wetland habitats on Bermuda. It is owned and managed by the Bermuda National Trust. 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 contains only two species of fish; *Fundulus bermudae* and a large number of mosquito fish. Red-eared sliders *Trachemys scripta elegans* are also present (Outerbridge, 2008). The killifish exist in very low abundance (Outerbridge, 2007a) so 20 adults (8 males and 12 females) were captured over a four month period in 2008 and translocated to a privately owned pond for captive breeding. The offspring were later translocated to Seymour's Pond (see below). The periphery of Warwick Pond is dominated by grasses and sedges. No mangrove species are present. Warwick Pond is extremely shallow; mean water depth is 20 cm and the maximum only 37 cm (Outerbridge and Thomas, unpublished data). However, Rueger (2004) reported that the organic sediment which forms the bottom of the pond is at least 8 feet deep. Water levels fluctuate as a result of rainfall and evaporation, but also as a result of long term tidal fluctuations raising and lowering the water table. The area of open water is greatly reduced during periods of low rainfall at which time mud flats appear at both ends of the pond. The average annual surface water temperatures are known to range from 10.8-39.6°C (mean 24.0°C) and salinities from 0-9.5 psu (mean 3.2 psu) (Outerbridge and Thomas, unpublished data).

Figure 21. 2012 aerial photograph of Warwick Pond

Riddell's Bay, Pond on the #15 fairway

This marine, femur bone-shaped pond (Fig. 22) was originally created as a water hazard on the Riddell's Bay golf course but management gave permission for *Fundulus bermudae* (n=100) to be introduced in 2012. The founding fish came from Evan's Pond; all were juveniles at the time of the translocation. An assessment of the new sub-population in 2013 revealed the presence of an estimated 417 fish, many of which were large adults (Outerbridge, unpublished data). The salinity of the pond at the time of the assessment (August) was 33 psu; however the underwater topology, as well as the annual variation in water temperature and salinity, has not been studied. There is very little vegetation surrounding the pond but widgeon grass *Ruppia maritima* grows abundantly within the pond.

Figure 22. 2012 aerial photograph of the pond on the 15th fairway of the Riddell's Bay golf course

Seymour's Pond

By the early 20th century approximately one third of the original freshwater pond had become filled with garbage. Encroachment by vegetation further reduced the area of open water. In 2011 the Bermuda Audubon Society re-excavated the pond, creating approximately 0.8 acres of open water and two small islands for waterfowl to nest on safely (Fig. 23). Salinity was measured after excavation and was found to be brackish (24 psu). *Fundulus bermudae* (n=400) were introduced into the pond a few months later. These fish were the offspring of the 20 adults captured from Warwick Pond in 2008. A survey of the Seymour's Pond sub-population in 2012 estimated that it had increased to 10,316 individuals (Copeland, 2013). The underwater topology, as well as the annual variation in water temperature and salinity, of this pond is unknown.

Figure 23. 2012 aerial photograph of Seymour's Pond

Sea Swept Farm Pond

This pond is situated on private land in Southampton Parish (Fig. 24). It was originally an enlarged drainage ditch where road water run-off was channeled during periods of rainfall; however the ditch was greatly enlarged in 2007 to create a half acre pond. 103 juvenile *Fundulus bermudae* were translocated from Evan's Pond into the Sea Swept Farm Pond in 2008. The translocation appears to have been successful because 26 adult males and females were captured in 2017 (A. Wolf, pers. comm.), therefore an assessment of this sub-population is desirable. The underwater topology, as well as the annual variation in water temperature and salinity of this pond is unknown.

Figure 24. 2012 aerial photograph of the pond on Sea Swept Farm

Evan's Pond

Evan's Pond is 1.79 acres and situated on private land in Southampton Parish (Fig. 25). The northern end is adjacent to a nature reserve owned and managed by the Buy Back Bermuda Committee. The pond is surrounded by a mixture of red and black mangroves and is a simple basin characterized by shallow depths, fairly even contours and a gently sloping shoreline. It 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). The mean depth in Evan's Pond is 65 cm and the maximum is 120 cm in the center of the pond. Average annual surface water temperatures ranged from 17-32°C (mean 24.6°C) and salinities varied from 28-44.5 psu (mean 34.1 psu) (Thomas et al., 1991). *Fundulus bermudae* inhabit this pond but have proved to be exceedingly difficult to trap, therefore population assessments have not been possible. Small schools of juvenile killifish are regularly seen in the summer and fall months at the northern end of the pond. A variety of marine fishes cohabit this pond with the killifish including gray snappers *Lutjanus griseus*, horse-eye jacks *Caranx latus*, great barracuda *Sphyraena barracuda*, shads *Eucinostomus spp*., breams *Diplodus bermudensis*, and liza mullets *Mugil liza*.

Figure 25. 2012 aerial photograph of Evan's Pond (left) and map of bathymetry (right)
(Depth is in cm. Black arrow indicates area of tidal exchange)
Bathymetry map adapted from Thomas et al., 1991

Madagascar Pond at the Bermuda Aquarium Museum and Zoo

The Madagascar exhibit opened to the public in 2011 and contains a waterfall and a fresh water pond (Fig. 26). *Fundulus bermudae* from the Wind Reach Pond (therefore Warwick Pond stock) were introduced into this pond in 2012 in order to generate a sub-population from which individuals could be easily captured and put on public display at BAMZ, thereby eliminating the need to collect specimens from the wild.

Figure 26. Photograph of the Madagascar pond at the Bermuda Aquarium Museum and Zoo