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Molecular characterization of nearshore baitfish populations in Bermuda to inform management

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Small-bodied marine fishes play an important role in the food web, feeding both larger fishes and seabirds. Often referred to as baitfishes, they concentrate seasonally in coastal areas in large, often heterospecific assemblages that are targeted by both commercial and recreational fishers. Given apparent declines in at least some of Bermuda's baitfish species over the past 40 years, it is useful to determine the species composition of baitfish assemblages, and how it varies among sites, in order to inform management. Using genetic barcoding of the Cytochrome c oxidase 1 gene (COI), we confirm species identity, assess intraspecific genetic diversity locally, and determine rates of broader genetic connectivity for baitfish assemblages in Bermuda. Species analyzed included *Hypoatherina harringtonensis*, *Anchoa choerostoma*, *Jenkinsia lamprotaenia*, *Harengula humeralis*, *Opisthonema oglinum* and *Sardinella aurita*. Species identification based on molecular barcoding revealed some misidentification of individuals based solely on morphological traits, with an error rate of 11%, validating the usefulness of this approach. Interestingly, sequence results for the endemic Bermuda anchovy, *A. choerostoma*, were within 1% similarity to the more broadly distributed big-eye anchovy, *A. lamprotaenia*, and thus additional analyses are warranted to evaluate the genetic basis for endemism. Estimates of genetic diversity within and among baitfish assemblages in Bermuda were high, indicating high rates of local connectivity among sites for all species. As such, management should consider Bermuda's baitfish species as single, highly mixed populations. However, with the exception of *H. humeralis* and the endemic *A. choerostoma*, significant genetic differentiation and population structure were found when comparing Bermuda's baitfish populations with conspecifics from other regions, suggesting limited gene flow between other regions and Bermuda for these species. Limited regional connectivity has implications for management, as strong genetic divergence suggests that

populations in Bermuda are predominantly self-seeding and thus not likely to be replenished from distant populations. These results therefore support precautionary management of baitfish species in Bermuda.

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Abstract

Small-bodied marine fishes play an important role in the food web, feeding both larger fishes and seabirds. Often referred to as baitfishes, they concentrate seasonally in coastal areas in large, often heterospecific assemblages that are targeted by both commercial and recreational fishers. Given apparent declines in at least some of Bermuda's baitfish species over the past 40 years, it is useful to determine the species composition of baitfish assemblages, and how it varies among sites, in order to inform management. Using genetic barcoding of the Cytochrome c oxidase 1 gene (COI), we confirm species identity, assess intraspecific genetic diversity locally, and determine rates of broader genetic connectivity for baitfish assemblages in Bermuda. Species analyzed included *Hypoatherina harringtonensis*, *Anchoa choerostoma*, *Jenkinsia lamprotaenia*, *Harengula humeralis*, *Opisthonema oglinum* and *Sardinella aurita*. Species identification based on molecular barcoding revealed some misidentification of individuals based solely on morphological ~~traits,~~ with an error rate of 11%, validating the usefulness of this approach. Interestingly, sequence results for the endemic Bermuda anchovy, *A. choerostoma*, were within 1% similarity to the more broadly distributed big-eye anchovy, *A. lamprotaenia*, and thus additional analyses are warranted to evaluate the genetic basis for endemism. Estimates of genetic diversity within and among baitfish assemblages in Bermuda were high, indicating high rates of local connectivity among sites for all species. As such, management should consider Bermuda's baitfish species as single, highly mixed populations. However, with the exception of *H. humeralis* and the endemic *A. choerostoma*, significant genetic differentiation and population structure were found when comparing Bermuda's baitfish populations with conspecifics from other regions, suggesting limited gene flow between other regions and Bermuda for these species. Limited regional connectivity has implications for management, as strong genetic

divergence suggests that populations in Bermuda are predominantly self-seeding and thus not likely to be replenished from distant populations. These results therefore support precautionary management of baitfish species in Bermuda.

Introduction

Small-bodied, shoaling marine fishes are a critical part of the food chain, connecting plankton at low trophic levels to higher trophic level organisms such as seabirds and piscivorous fishes (Smith et al., 2011; Pikitch et al., 2014). These species form large aggregations in coastal areas, and here they are targeted by both commercial and recreational fishers (Smith-Vaniz, Collette & Luckhurst, 1999; Smith et al., 2011). Commonly referred to as 'forage fish' or 'baitfish', their combined ecological and fisheries importance makes these species a priority for management (Smith et al., 2011; Pikitch et al., 2014).

In Bermuda, large, heterospecific baitfish aggregations typically include several morphologically similar species from the families Clupeidae, Engraulidae, Atherinidae, and Hemiramphidae (Parrish, 1989; Smith-Vaniz, Collette & Luckhurst, 1999). Species frequently targeted by fishermen include the Reef silverside, *Hypoatherina harringtonensis* (G.B. Goode, 1877) [Family Atherinidae], the endemic Bermuda anchovy, *Anchoa choerostoma* (G.B. Goode, 1874) [F. Engraulidae], and the Dwarf herring, *Jenkinsia lamprotaenia* (P.H. Gosse, 1851) [F. Dussumieriidae], as well as the larger and morphologically similar Redear herring, *Harengula humeralis* (G. Cuvier, 1829), Round sardine, *Sardinella aurita* (A. Valenciennes, 1847), and Threadfin herring, *Opisthonema oglinum* (C.A. Lesueur, 1818) [F. Clupeidae] (Smith-Vaniz, Collette & Luckhurst, 1999; Lavoué, Konstantinidis & Chen, 2014).

Targeted commercial baitfish fisheries in Bermuda utilize seine nets to harvest shoaling fish, which are then used secondarily as either line bait or chum in further fishing activities, or sold to recreational fishers. Recreational fishers may use a cast net only to catch bait for personal use (Bermuda Fisheries Regulations 2010). Prior to the banning of fish traps in 1990, these small, oily fishes were also placed in mesh bags that were added to traps in order to draw larger fishes to them from greater distances (Butler et al., 1993; Smith-Vaniz, Collette & Luckhurst, 1999). The annual harvest of baitfishes in Bermuda peaked at 105,072 kg in 1988, corresponding with the peak of local trap fishing activity, and fell to a low of 26,842 kg in 1995 (Butler et al., 1993; Smith-Vaniz, Collette & Luckhurst, 1999). Long-term landings statistics show that, after a decade of adjustment, total reported commercial catches of baitfish species have remained largely stable in the range of 30,000 - 40,000 kg per annum between 1999 and 2017 (Bermuda Government Department of Environment and Natural Resources, unpubl. data). The Dwarf herring and Bermuda anchovy, which, along with the Reef silverside are collectively called 'fry', make up the bulk of the baitfish harvest (Smith-Vaniz, Collette & Luckhurst, 1999). However, anecdotal evidence from fishers, together with landings records for the larger baitfish species, suggests that local populations of at least some species have declined over the past 40 years.

For species targeted by fishing, overexploitation can lead to population declines (Ecoutin et al., 2010; Last et al., 2010; Stagličić et al., 2011). Other anthropogenic impacts in coastal areas, such as pollution and habitat degradation (Kennish, 2002; Hewitt et al., 2008; Johnston & Roberts, 2009), can also alter fish distribution patterns, decreasing richness and abundance across various spatial and temporal scales (Sax & Gaines, 2003; Johnston & Roberts, 2009). Apparent declines in the abundance of baitfishes in Bermuda may therefore reflect natural fluctuations in the abundance and distribution of these species, or may be indicative of

overfishing or other anthropogenic impacts. However, a significant change in fishing practices, such as the banning of fish traps, may also affect the ways in which a related resource, such as bait, is harvested and used, which may in turn affect how that resource is perceived and monitored by fishers.

At present, regulation of baitfishing in Bermuda under the Fisheries Act, 1972, and the Fisheries Regulations, 2010, includes both gear and spatial restrictions. In particular, there are four inshore bays (Somerset Long Bay, Shelly Bay, Whalebone Bay, and Coot Pond), within which the use of fishing nets and the removal of baitfishes is completely prohibited (Bermuda Fisheries Act, 1972, Section 8i), but these bays are not evenly distributed around the island (Fig. 1). Further, given the similarities in species morphologies, visual identification of baitfish species can be difficult and a higher diversity may exist within baitfish schools than is currently perceived. Thus, a greater understanding of how assemblage composition varies across locations, along with the extent of local movements and genetic mixing, is required to inform management.

Lastly, if Bermuda's baitfish populations are indeed experiencing declines, a greater understanding of regional genetic connectivity could indicate whether or not larval supply from other populations might assist with their recovery (see Cowen & Sponaugle, 2009). Bermuda's isolated, mid-Atlantic location (Fig. 1, inset) reduces the likelihood of regular supply of larvae from external sources (Schultz & Cowen, 1994), and there is evidence indicating that at least some local fish populations are self-seeding (see Locke et al., 2013). However, despite this isolation, Bermuda was the first location outside of the eastern coast of the United States where invasive lionfish were detected (Whitfield et al., 2002), indicating that larval transport and / or post-larval rafting in association with floating material across this distance is not only possible but occurs at a rate that enabled successful establishment of an invasive species (Locke et al.,

2013). Further, Bermuda has low rates of endemism (Smith-Vaniz, Collette & Luckhurst, 1999), suggesting at least some genetic connectivity with other regions (see Locke et al., 2013).

Using genetic barcoding of the Cytochrome c oxidase 1 gene (COI), we aim to confirm species identity, assess intraspecific genetic diversity, and determine rates of local and regional genetic connectivity of Bermuda's baitfish populations. Barcoding has proven useful for species identification where specimens cannot be distinguished based on morphology, and has been able to identify new species by integrative taxonomic analysis (Ward, Hanner & Hebert, 2009). Furthermore, population genetic analyses can suggest points of origin in mixed populations and provide insights to breeding structures (Allendorf & Utter, 1979). Thus, results of this study will provide insights into population stability and can be used to inform future management strategies.

Materials & Methods

Samples were collected from 10 locations around the islands of Bermuda: the Bermuda Aquarium Museum and Zoo dock in Flatts (BAMZ), Bailey's Bay, Coney Island, Whalebone Bay, Turtle Bay, South Bay, East Whale Bay, West Whale Bay, Frank's Bay, and Deep Bay, between July and August 2017 (Fig. 1 – clockwise from center). All samples were collected with permission of the Bermuda Government, Department of Environment and Natural Resources under special permit SP170303. A total of 111 individuals were collected and visually identified based on previously described morphological characteristics (Smith-Vaniz, Collette & Luckhurst, 1999). Based on initial morphological examinations, five species were identified: *Hypoatherina harringtonensis* (Reef silverside), *Anchoa choerostoma* (Bermuda anchovy), *Jenkinsia lamprotaenia* (Dwarf herring), *Harengula humeralis* (Redear herring) and *Sardinella*

aurita (Round sardinella). Representative samples of each species from each location were preserved in 95% ethanol for subsequent genetic analyses.

Genomic DNA was extracted from muscle tissue of samples using a Qiagen DNA Blood and Tissue extraction kit following the manufacturers protocols, resulting in a final volume of 200 µl. The cytochrome c oxidase (COI) gene was amplified from extracted DNA using a primer cocktail developed for fish barcoding as described by Ivanova et al. (2007) (COI-3: C_FishF1t1-C_FishR1t1). All PCRs had a total volume of 12.5 µl and included: 6.25 µl of 5% DMSO, 2.00 µl of H_2O, 1.25 µl of 10x Buffer [10mM KCl, 10mM $(NH_4)SO_4$, 20 mM Tris-HCl (pH 8.8), 2mM $MgSO_4$, 0.1% Triton X-100], 0.625 µL $MgCl_2$ (50mM), 0.125 µl of each primer cocktail, 0.0625 µl of DNTP (10 mM), 0.0625 µl of *Taq* Polymerase (Invitrogen), and 2 µl of DNA template. PCR was optimized at the following: 95°C for 2 minutes, 35 cycles of 94 °C for 30 seconds, 52°C for 30 seconds, 72°C for 1 minute, with a final extension at 72°C for 10 minutes. PCR products were visualized using a 1.2% agarose gel, with concentration and purity measured using a spectrophotometer. Products were bi-directionally sequenced with universal M13 primers using Sanger Sequencing services provided by Gene Codes Corporation. Resulting sequences were manually edited and aligned using Sequencher® 5.4.6 (Gene Codes Corporation, Ann Arbor, MI, USA) and compared to known sequences in NCBI Blast and GenBank. Accession numbers are listed in Appendix 1, all sequences are available on GenBank (www.ncbi.nlm.nih.gov/Genbank). Available sequences of the COI gene for conspecifics from locations outside Bermuda were downloaded from GenBank and used for regional comparisons of genetic structure and connectivity (Valdez-Moreno et al., 2010; Lavoué, Konstantinidis & Chen, 2014). Sequences from *Anchoa lamprotaenia* [Hildebrand, 1943] from Florida (Weigt et

al., 2012; Lavoué, Konstantinidis & Chen, 2014) were used for comparisons with *A. choerostoma*, as no COI sequences were available for *A. choerostoma*.

Sequences for all species, including those obtained from GenBank, were aligned using MUSCLE (Edgar, 2004). Molecular phylogenetic analysis to infer evolutionary history was conducted by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei, 1993). Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 154 nucleotide sequences, with a total of 718 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

Diversity was assessed within and among locations in Bermuda using standard diversity indices, including number of haplotypes (*Nh*), number of polymorphic sites (*Np*), haplotypic diversity (*h*) (Nei, 1987), nucleotide diversity (*pn*) (Tajima, 1983; Nei, 1987), and mean number of pairwise differences (*pd*) between haplotypes (Tajima, 1983) calculated for each species using DnaSP v.5 (Librado & Rozas, 2009). Population (location) pairwise F_{ST} (Hudson, Slatkin & Maddison, 1992) values, whose significances were assessed through 10,000 permutation tests, were used to calculate differentiation between locations within Bermuda, as well between Bermuda and other regional locations, using ARLEQUIN version 3.5.2 (Excoffier & Lischer, 2010). Unweighted analysis of molecular variance (AMOVA; (Excoffier, Smouse & Quattro, 1992) was also performed to test hierarchical models of genetic variance using pairwise differences among haplotypes as a measure of divergence within and among locations in

Bermuda as well as within and among regional locations using ARLEQUIN version 3.5.2 (Excoffier & Lischer, 2010).

Results

A total of 92 individual fish were successfully sequenced (see Appendix 1). Alignments for each species were trivial and required no insertion / deletion events. Sequence identification of 81 individuals confirmed morphological identification, while sequence and morphological identification did not match for 10 individuals, indicating an error rate of 11% for identification based on morphology.

All three samples from Deep Bay, initially identified as *Sardinella aurita*, were molecularly identified as *Opisthonema oglinum*. Five of the seven samples from the BAMZ location that were initially classified as *S. aurita* were also molecularly identified as *O. oglinum*. One sample from Frank's Bay and one sample from Coney Island were morphologically identified as *Hypoatherina harringtonensis*, but resulting sequences matched that of *Jenkinsia lamprotaenia.* COI sequences of individuals morphologically identified as the endemic Bermuda anchovy, *Anchoa choerostoma*, had a 99% identity match (93% query coverage) to the widespread Atlantic species *A. lamprotaenia*. However, no COI sequences were available for *A. choerostoma* on public databases for comparison.

The phylogenetic tree constructed based on maximum likelihood (Fig. 2) indicates that species from the genera *Harengula, Opisthonema*, and *Sardinella* are more closely related to each other than to the other species examined, while species from the genera *Jenkinsia* and *Anchoa* are more closely related to each other than to the other species. The genus *Hypoatherina*, in the order Atheriniformes, was the most evolutionarily distant from the other genera.

Haplotype diversity was similar among species based on overlapping standard errors (Table 1). Nucleotide diversity was similar for *A. choerostoma* and *H. harringtonensis*, and for *H. humeralis, J. lamprotaenia*, and *O. oglinum,* but was higher for the first two species than for the latter three. The mean number of pairwise differences was highest for *A. choerostoma* at 3.163, decreasing to 0.934 for *J. lamprotaenia,* 0.800 for *H. humeralis,* 0.780 for *H. harringtonensis,* and 0.429 for *O. oglinum*. Diversity of *A. choerostoma* within a given bay was higher at East Whale Bay than at West Whale Bay and BAMZ (Table 1). For *H. harringtonensis*, within-location diversity was higher at BAMZ and Whalebone Bay than at Frank's Bay and Bailey's Bay. Diversity was similar among locations for *H. humeralis* at BAMZ and Coney Island, and among all sampled locations for *J. lamprotaenia*. Likewise, diversity measures did not differ among locations for *O. oglinum* from Deep Bay and BAMZ. While measures of diversity were high for *S. aurita,* the low sample size (n=2) and lack of replicate sites precludes their inclusion in diversity comparisons (Table 1).

Pairwise F_{ST} comparisons between locations within Bermuda were insignificant (α=0.05) for all species examined, indicating no evidence of genetic structure and high levels of genetic connectivity within and among locations (Table 2). An analysis of molecular variance used to test for hierarchical population structure also indicated no significant genetic structure exists among locations in Bermuda for any of the species analyzed, where the majority of variation for all species was found within locations rather than among them (Table 3).

For all species except *H. humeralis*, pairwise F_{ST} comparisons were significant (α=0.05) between populations from Bermuda and those from other regions, indicating strong evidence of genetic structure and limited genetic connectivity across regions (Table 4). An analysis of molecular variance, used to test for hierarchical population structure, also showed significant

genetic structure among regions for all species analyzed with the exception of *H. humeralis*, such that the majority of variation was found among regions rather than within them (Table 5).

Discussion

Based on the COI sequences obtained from baitfish samples in this study, a higher diversity of species was present within the assemblages than was initially recorded based solely on morphological identification, at an error rate of 11%. Genetic sequencing revealed the misidentification of several small individuals of *Opisthonema oglinum* that had yet to develop their distinctive threadfin and were thus misidentified as *Sardinella aurita*. As a result, the total number of species analyzed increased from five to six, and included *Hypoatherina harringtonensis*, *Anchoa choerostoma*, *Harengula humeralis*, *Jenkinsia lamprotaenia*, *O. oglinum,* and *S. aurita*. These results highlight the importance of incorporating molecular identification into assessments of species assemblages for morphologically similar species, as diversity may be underestimated when based only on morphology (Zemlak et al., 2009; Hubert et al., 2012).

The evolutionary relationship among the six genera of baitfishes examined in this study was analyzed using a Maximum Likelihood phylogenetic approach and concurs with the evolutionary relationships described by Lavoué, Konstantinidis & Chen (2014) (Fig. 2). Genera within the Family Clupeidae (*Harengula, Opisthonema,* and *Sardinella*), were found to be more closely related to each other than to the other genera examined. Likewise, *Jenkinsia* (Family Dussumieriidae) and *Anchoa* (Family Engraulidae) were found to be more closely related to each other than to the representative species from other genera. All of the aforementioned genera belong to the Order Clupeiformes, while *Hypoatherina* belongs to the Order Atheriniformes.

Accordingly, *H. harringtonensis* was found to be the most evolutionarily distant from the other genera examined.

Interestingly, the CO1 sequence results for the endemic Bermuda Anchovy, *A. choerostoma*, were within 1% similarity to the more broadly distributed Big-eye anchovy, *A. lamprotaenia*. *A. choerostoma* was described by Goode in 1874 based on morphological variations from congeneric species, and is most closely related, morphologically, to *A. lamprotaenia, A. januaria, A. cubana* and *A. parva* (Smith-Vaniz, Collette & Luckhurst, 1999; Nizinski & Munroe, 2002; Li & Ortí, 2007; Smith-Vaniz & Collette, 2013). *A. choerostoma* is distinguished from *A. mitchilli* by the relative positions of the dorsal fin and anal fin (such that the anal fin in *A. choerostoma* is posterior to the dorsal, whereas the origins of these fins are vertically aligned in *A. mitchilli*); from *A. lamprotaenia* by having a greater number of lower gill rakers (23 - 30 as opposed to 17-21); and from the remaining similar congenerics by having a notably smaller axillary scale above the pectoral fin (Smith-Vaniz, Collette & Luckhurst, 1999; Nizinski & Munroe, 2002).

Cytochrome c oxidase is an enzyme in the respiratory chain that catalyzes the conversion of oxygen to water, a critical survival process. Encoded inside mitochondria, the Cytochrome c oxidase subunit 1 gene (COI) is highly conserved among all respiring organisms and is therefore not subject to selective pressures that induce mutation (Mick, Fox & Rehling, 2011). It is possible, therefore, that the COI gene may not provide high enough resolution to distinguish between these closely related species. To further evaluate and obtain an accurate assessment of endemism, multiple genes should be incorporated and compared among several congeneric species. Coupled with morphological variation, detailed phylogenetic analyses could provide further insights into the classification of the Bermuda anchovy.

Of the locations examined around Bermuda, BAMZ had the highest species diversity, with all six species found at this location (Fig. 1). Conversely, at several locations, only a single species was found. However, this may reflect sampling effort rather than actual diversity, as these sites were sampled less frequently than BAMZ. Yet, BAMZ is also the most centrally located site on the more protected northern shore of Bermuda, and may, therefore, represent an area of species accumulation (Tittensor et al., 2010), at least for inshore species, that results in higher baitfish diversity. Among the species, *J. lamprotaenia* was the most widely distributed, being found at five of the 10 locations, followed by *H. harringtonensis* at four of the 10 locations. Given the high estimates of connectivity among locations for all six species examined, however, distributions are likely wider than reflected by the somewhat limited sampling effort in this study.

Estimates of genetic diversity within and among baitfish assemblages in Bermuda indicate high degrees of mixing between locations for all six species examined. For these small-bodied species, this mixing likely occurs predominantly during the larval phase (Schultz, 2000; Lavoué, Konstantinidis & Chen, 2014), but may also occur during later life stages as a result of short- or long-term movements between locations that may be driven by food availability, predator density, reproductive cycles or adverse conditions (Hugie & Dill, 1994; Olsson et al., 2006; Udyawer et al., 2013; Currey et al., 2015). These high rates of local connectivity mean that management should consider Bermuda's baitfish species as single, highly mixed populations. As such, the distribution of bays that are closed to net fishing in order to protect them is of less importance than it might be if subregional genetic differentiation had been detected, and there is no immediate need to close additional bays in central or western parishes, or along the south shore, in order to maintain local genetic diversity.

While baitfish families typically have a global distribution (Lavoué et al., 2013, 2017; Lavoué, Konstantinidis & Chen, 2014), most species are restricted to one of the world's marine biogeographic provinces (Spalding et al., 2007; Briggs & Bowen, 2011), with high rates of endemism occurring in tropical regions (Lavoué et al., 2013). Restricted geographical distribution and high rates of endemism indicate low dispersal capabilities for these species, with inter-regional distribution likely constrained by vast expanses of open ocean and water temperature (Lavoué et al., 2013). Most of the species occurring in Bermuda are restricted to the Tropical Northwestern Atlantic province (Spalding et al., 2007; Locke et al., 2013), which is largely equivalent to the Caribbean biogeographic province (*sensu* Briggs & Bowen, 2013). However, *S. aurita* is found on both sides of the Atlantic Ocean and into the Mediterranean Sea (Aquamaps, 2016).

The present study documents significant genetic differentiation between conspecific populations of baitfishes in Bermuda and those in other regions (Table 4; F_{ST}; $p<0.05$) and significant divergence among regions (Table 5; AMOVA; $p<0.05$) for *J. lamprotaenia*, *H. harringtonensis*, *O. oglinum,* and *S. aurita,* as well as between congeneric populations of *Anchoa sp.* among regions. These results further suggest that gene flow, and therefore exchange of individuals, is limited between populations of these species in Bermuda and those in other regions. Populations of *H. humeralis*, on the other hand, show no evidence of genetic structure, suggesting broad genetic connectivity exists across the Caribbean / Tropical West Atlantic for this species. Importantly, these results have major implications for management as strong genetic divergence in *J. lamprotaenia*, *H. harringtonensis*, *O. oglinum,* and *S. aurita* suggests that populations of these species in Bermuda are likely self-seeding and locally maintained. Thus, local declines in baitfish abundances are not likely to be replenished from distant populations.

312

313 **Conclusion**

314 Baitfishes depend on shallow inshore areas, which are capable of sustaining great
315 diversity and densities of organisms (Nagelkerken et al., 2001; Ray & Carleton Ray, 2005;
316 Vasconcelos et al., 2011; Araújo et al., 2017), but are also extensively modified and threatened
317 by human activities such as overfishing, pollution, coastal development and habitat degradation,
318 which may impact fish communities (Kennish, 2002; Sax & Gaines, 2003; Ribeiro et al., 2008;
319 Johnston & Roberts, 2009; Ecoutin et al., 2010; Last et al., 2010; Araújo et al., 2017). The
320 limited genetic connectivity of baitfish populations among Western Atlantic regions documented
321 here indicates restricted influx of new individuals to Bermuda and highlights the vulnerability of
322 local populations to natural and anthropogenic perturbations. As such, it is important to monitor
323 both fish communities and environmental parameters in Bermuda's nearshore habitats (Araújo et
324 al., 2017), and to adapt management measures accordingly, in order to conserve these
325 ecologically and economically important species.

326

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330

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Table 1(on next page)

Diversity Measures.

Standard diversity measures by sampling location for *A. choerostoma, H. harringtonensis, H. humeralis, J. lamprotaenia, O. oglinum,* and *S. aurita* including sample size (*n*), number of usable base pairs (*bp*), number of haplotypes (*Nh*), number of polymorphic sites (*Np*), haplotype diversity (*h*), nucleotide diversity (*pn*), and the mean number of pairwise differences (*pd*).

Species	Location	*n*	*bp*	*Nh*	*Np*	*h*	*pn*	*pd*
A. choerostoma	ALL	20	710	11	16	0.763 +/- 0.103	0.0045 +/- 0.0010	3.163
	West Whale Bay	4	710	2	1	0.500 +/- 0.265	0.0007 +/- 0.0004	0.500
	East Whale Bay	8	710	8	12	1.00 +/- 0.0039	0.0062 +/- 0.0010	4.429
	BAMZ	8	710	3	8	0.464 +/- 0.040	0.0043 +/- 0.0018	3.071
H. harringtonensis	ALL	25	718	9	8	0.640 +/- 0.107	0.00295 +/- 0.000002	0.780
	Whalebone Bay	8	718	4	3	0.750 +/- 0.139	0.0013 +/- 0.0016	0.929
	Frank's Bay	7	718	2	1	0.286+/- 0.196	0.0006 +/- 0.0006	0.286
	Bailey's Bay	2	718	1	0	0	0.00	0
	BAMZ	8	718	6	5	0.893 +/- 0.111	0.0027 +/- 0.0016	1.25
H. humeralis	ALL	10	707	5	4	0.667 +/- 0.163	0.0011 +/- 0.0004	0.800
	BAMZ	6	707	4	3	0.800 +/- 0.172	0.0014 +/- 0.0004	1.000
	Coney Island	4	707	2	1	0.500 +/- 0.265	0.0007 +/- 0.0004	0.500
J. lamprotaenia	ALL	27	718	10	9	0.650 +/- 0.103	0.0013 +/- 0.0003	0.934
	South Bay	8	718	3	3	0.607 +/- 0.164	0.0013 +/- 0.0005	0.929
	Coney Island	9	718	5	4	0.722 +/- 0.159	0.0015 +/- 0.0005	1.056
	Frank's Bay	1	718	1	0	0	0	0
	Turtle Bay	1	718	1	0	0	0	0
	BAMZ	8	718	5	4	0.786 +/- 0.151	0.0014 +/- 0.0004	1.000
O. oglinum	ALL	8	709	2	1	0.429 +/- 0.169	0.0006 +/- 0.0002	0.429
	Deep Bay	3	709	2	1	0.667 +/- 0.314	0.0009 +/- 0.0004	0.667
	BAMZ	5	709	2	1	0.400 +/- 0.237	0.0006 +/- 0.0003	0.400
S. aurita	BAMZ	2	681	2	3	1.000 +/- 0.500	0.0044 +/- 0.0022	3.000

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Table 2(on next page)

Genetic Connectivity within Bermuda.

Pairwise F_{ST} values among sampling locations for each species. Significant comparisons are indicated in bold (α = 0.05).

A. choerostoma	West Whale Bay	East Whale Bay	BAMZ		
West Whale Bay	0				
East Whale Bay	0.07556	0			
BAMZ	0.01604	-0.05263	0		
H. harringtonensis	Whalebone Bay	Frank's Bay	Bailey's Bay	BAMZ	
Whalebone Bay	0				
Frank's Bay	0.04465	0			
Bailey's Bay	-0.24551	-0.3125	0		
BAMZ	-0.02521	-0.01165	-0.31765	0	
H. humeralis	Coney Island	BAMZ			
Coney Island	0				
BAMZ	-0.0297	0			
J. lamprotaenia	South Bay	Coney Island	Frank's Bay	Turtle Bay	BAMZ
South Bay	0				
Coney Island	0.00697	0			
Frank's Bay	-0.85714	-0.9	0		
Turtle Bay	-0.85714	-0.9	0	0	
BAMZ	0.00461	0.02589	-1	-1	0
O. oglinum	Deep Bay Beach	BAMZ			
Deep Bay	0				
BAMZ	-0.29921	0			

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Table 3(on next page)

AMOVA within Bermuda.

Analysis of molecular variance within and among sampled locations around Bermuda (Arlequin 3.5.2). Samples from all locations for a given species were considered as a single group. Significant F_{ST} indices are indicated in bold (α=0.05).

Species	Source of Variation	d.f.	Sum of Squares	Variance components	Percentage of total variation	Fixation indices
A. choerostoma						
	Among locations	2	3.05	- 0.00988 Va	- 0.63	F_{ST}: - 0.00626
	Within locations	17	27.00	1.58824 Vb	100.63	
	Total	19	30.05	1.57835		
H. harringtonensis						
	Among locations	3	0.88	- 0.01880 Va	- 4.88	F_{ST}: - 0.04882
	Within locations	21	8.48	0.40391 Vb	104.88	
	Total	24	9.36	0.38511		
H. humeralis						
	Among locations	1	0.35	- 0.01172 Va	- 2.97	F_{ST}: - 0.02970
	Within loclations	8	3.25	0.40625 Vb	102.97	
	Total	9	3.60	0.39453		
J. lamprotaenia						
	Among locations	4	1.18	- 0.04269 Va	- 9.36	F_{ST}: - 0.09361
	Within locations	22	10.97	0.49874 Vb	109.36	
	Total	16	12.15	0.45605		
O. oglinum						
	Among locations	1	0.03	- 0.05630 Va	- 29.92	F_{ST}: - 0.29921
	Within locations	6	1.47	0.24444 Vb	129.92	
	Total	7	1.50	0.18815		

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Table 4(on next page)

Regional Genetic Connectivity.

Pairwise F_{ST} values among regions for each species. Significant comparisons are indicated in bold ($\alpha = 0.05$).

A. choerostoma	n	Bermuda	Florida	
Bermuda	20	0		
Florida (*A. lamprotaenia*)	5	**0.46211**	**0**	
H. harringtonensis	n	Bermuda	Belize	
Bermuda	25	0		
Belize	2	**0.97053**	**0**	
H. humeralis	n	Bermuda	Mexico	
Bermuda	10	0		
Mexico	4	0.08142	0	
Belize	2	-0.32353	-0.26316	0
J. lamprotaenia	n	Bermuda	Mexico	Belize
Bermuda	27	0		
Mexico	17	**0.98389**	0	
Belize	5	**0.92381**	**0.92381**	0
O. oglinum	n	Bermuda	Mexico	Brazil
Bermuda	8	0		
Mexico	4	**0.56408**	0	
Brazil	6	**0.69100**	0.15109	0
S. aurita	n	Bermuda	Turkey	Israel
Bermuda	2	0		
Turkey	3	**0.68902**	0	
Israel	6	**0.80840**	-0.05882	0

1

Table 5(on next page)

AMOVA Among Regions.

Analysis of molecular variance among and within regions (Arlequin 3.5.2). Populations from all regions for a given species were considered as a single group. Significant F_{ST} indices are indicated in bold (α=0.05).

Species	Source of Variation	d.f.	Sum of Squares	Variance components	Percentage of total variation	Fixation indices
A. choerostoma / lamprotaenia						
	Among regions	1	9.67	1.05522 Va	46.21	**F_{ST}: 0.46211**
	Within regions	23	28.25	1.22826 Vb	53.79	
	Total	14	37.92	2.28348		
H. harringtonensis						
	Among regions	1	46.05	12.33171 Va	97.05	**F_{ST}: 0.97053**
	Within regions	25	9.36	0.37440 Vb	2.95	
	Total	26	55.41	12.70611		
H. humeralis						
	Among regions	2	0.90	- 0.01357 Va	-2.75	F_{ST}: - 0.02747
	Within regions	13	6.60	0.50769 Vb	102.75	
	Total	15	7.50	0.49412		
J. lamprotaenia						
	Among regions	2	1061.95	38.14984 Va	94.25	**F_{ST}: 0.94252**
	Within regions	46	107.03	2.32675 Vb	5.75	
	Total	48	1168.98	40.47659		
O. oglinum						
	Among regions	2	14.53	1.09471 Va	53.83	**F_{ST}: 0.53831**
	Within regions	15	14.08	0.93889 Vb	46.17	
	Total	17	28.61	2.0336		
S. aurita						
	Among regions	2	8.23	1.08507 Va	65.86	**F_{ST}: 0.65859**
	Within regions	8	4.50	0.56250 Vb	34.14	
	Total	10	12.73	1.64757		

1

Figure 1

Map of Bermuda.

Map indicating the locations of bays that are currently closed to net fishing (red circles with strikethrough) and of sampled baitfish populations (pie charts). Size of pie charts represents the total number of individuals sequenced from that location. Colors within the circles represent the relative abundance of each species found at each location. The inset shows the isolated location of Bermuda within the west central Atlantic.

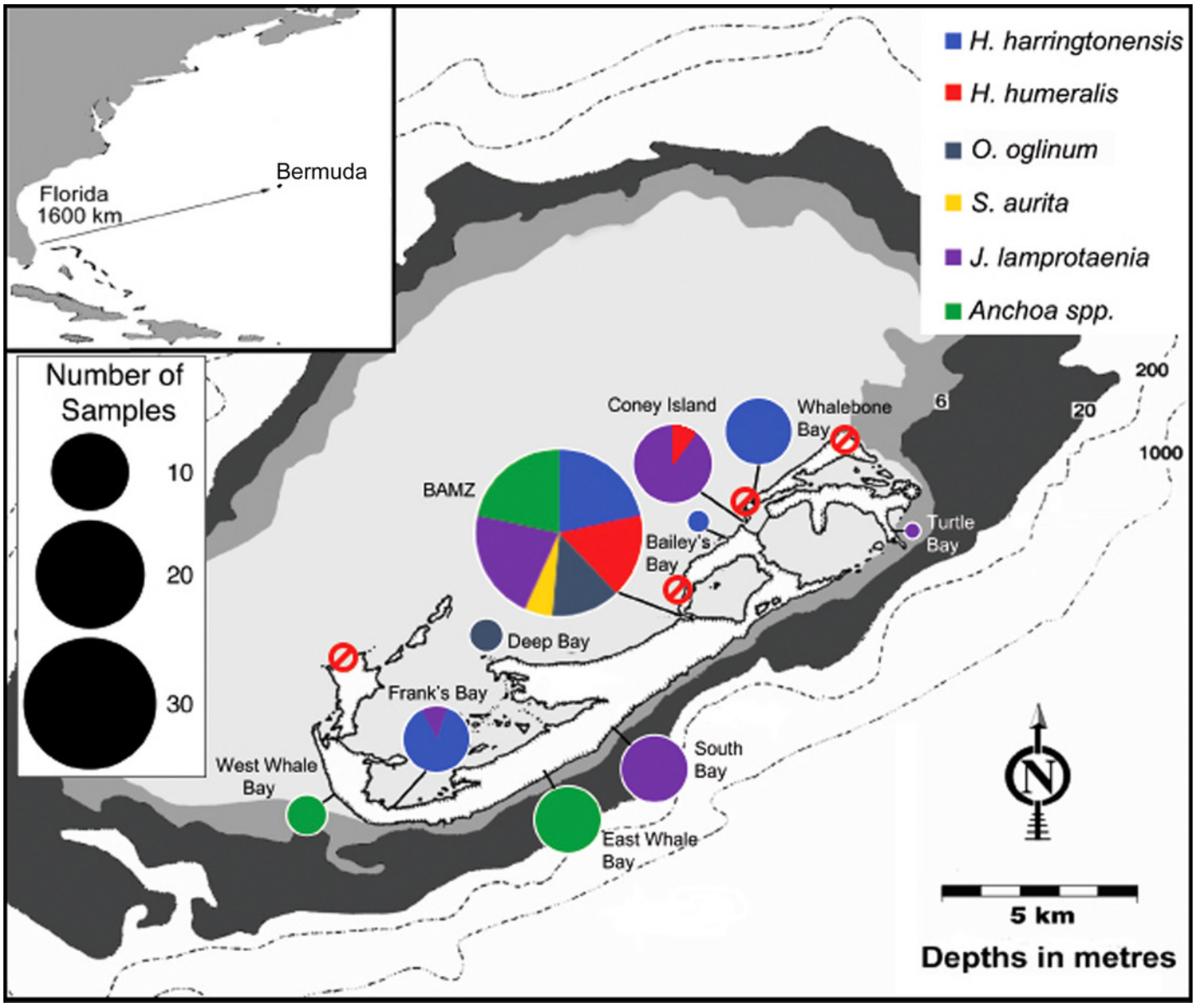
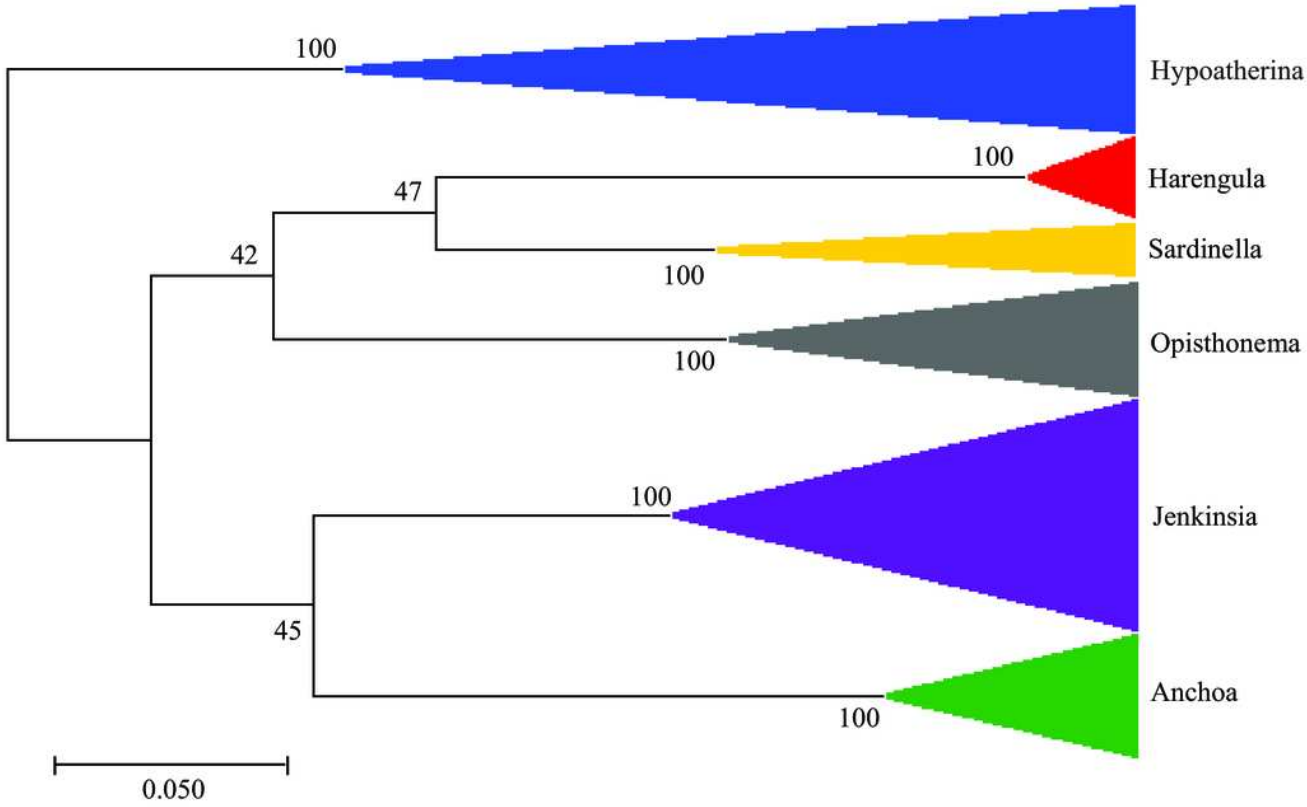

Florida
1600 km
Bermuda
H. harringtonensis
H. humeralis
O. oglinum
S. aurita
J. lamprotaenia
Anchoa spp.
Number of Samples
10
20
30
Coney Island
Whalebone Bay
BAMZ
Bailey's Bay
Turtle Bay
Deep Bay
Frank's Bay
West Whale Bay
South Bay
East Whale Bay
6
20
200
1000
N
5 km
Depths in metres

Figure 2

Molecular Phylogenetic Analysis.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). The tree with the highest log likelihood (-4350.82) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths in the number of substitutions per site. The analysis involved 154 nucleotide sequences. There were a total of 718 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al 2018).


100
Hypoatherina
100
47
Harengula
42
Sardinella
100
100
Opisthonema
100
Jenkinsia
45
Anchoa
100
0.050