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 gross morphological characteristics, 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 those 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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16 **Abstract**

17   Small-bodied marine fishes play an important role in the food web, feeding both larger

18 fishes and seabirds. Often referred to as baitfishes, they concentrate seasonally in coastal areas in

19 large, often heterospecific assemblages that are targeted by both commercial and recreational

20 fishers. Given apparent declines in at least some of Bermuda's baitfish species over the past 40

21 years, it is useful to determine the species composition of baitfish assemblages, and how it varies

22 among sites, in order to inform management. Using genetic barcoding of the Cytochrome c

23 oxidase 1 gene (COI), we confirm species identity, assess intraspecific genetic diversity locally,

24 and determine rates of broader genetic connectivity for baitfish assemblages in Bermuda. Species

25 analyzed included *Hypoatherina harringtonensis*, *Anchoa choerostoma*, *Jenkinsia lamprotaenia*,

26 *Harengula humeralis*, *Opisthonema oglinum* and *Sardinella aurita*. Species identification based

27 on molecular barcoding revealed some misidentification of individuals based solely on gross

28 morphological characteristics, with an error rate of 11%, validating the usefulness of this

29 approach. Interestingly, sequence results for the endemic Bermuda anchovy, *A. choerostoma*,

30 were within 1% similarity to the more broadly distributed big-eye anchovy, *A. lamprotaenia*, and

31 thus additional analyses are warranted to evaluate the genetic basis for endemism. Estimates of

32 genetic diversity within and among baitfish assemblages in Bermuda were high, indicating high

33 rates of local connectivity among sites for all species. As such, management should consider

34 Bermuda's baitfish species as single, highly mixed populations. However, with the exception of

35 *H. humeralis* and the endemic *A. choerostoma*, significant genetic differentiation and population

36 structure were found when comparing Bermuda's baitfish populations with those from other

37 regions, suggesting limited gene flow between other regions and Bermuda for these species.

38 Limited regional connectivity has implications for management, as strong genetic divergence

39   suggests that populations in Bermuda are predominantly self-seeding and thus not likely to be

40   replenished from distant populations. These results therefore support precautionary management

41   of baitfish species in Bermuda.

42

43   **Introduction**

44   Small-bodied, shoaling marine fishes are a critical part of the food chain, connecting

45   plankton at low trophic levels to higher trophic level organisms such as seabirds and piscivorous

46   fishes (Smith et al., 2011; Pikitch et al., 2014). These species form large aggregations in coastal

47   areas, and here they are targeted by both commercial and recreational fishers (Smith-Vaniz,

48   Collette & Luckhurst, 1999; Smith et al., 2011). Commonly referred to as 'forage fish' or

49   'baitfish', their combined ecological and fisheries importance makes these species a priority for

50   management (Smith et al., 2011; Pikitch et al., 2014).

51   In Bermuda, large, heterospecific baitfish aggregations typically include several

52   morphologically similar species from the families Clupeidae, Engraulidae, Atherinidae, and

53   Hemiramphidae (Parrish, 1989; Smith-Vaniz, Collette & Luckhurst, 1999). Species frequently

54   targeted by fishermen include the Reef silverside, *Hypoatherina harringtonensis* (G.B. Goode,

55   1877) [Family Atherinidae], the endemic Bermuda anchovy, *Anchoa choerostoma* (G.B. Goode,

56   1874) [F. Engraulidae], and the Dwarf herring, *Jenkinsia lamprotaenia* (P.H. Gosse, 1851) [F.

57   Dussumieriidae], as well as the larger and morphologically similar Redear herring, *Harengula*

58   *humeralis* (G. Cuvier, 1829), Round sardinella, *Sardinella aurita* (A. Valenciennes, 1847), and

59   Threadfin herring, *Opisthonema oglinum* (C.A. Lesueur, 1818), all [F. Clupeidae] (Smith-Vaniz,

60   Collette & Luckhurst, 1999; Lavoué, Konstantinidis & Chen, 2014).

61   Targeted commercial baitfish fisheries in Bermuda utilize seine nets to harvest shoaling

62   fish, which are then used secondarily as either line bait or chum in further fishing activities, or

63   sold to recreational fishers. Recreational fishers may use a cast net only to catch bait for personal

64   use (Bermuda Fisheries Regulations 2010). Prior to the banning of fish traps in 1990, these

65   small, oily fishes were also placed in mesh bags that were added to traps in order to draw larger

66   fishes to them from greater distances (Butler et al., 1993; Smith-Vaniz, Collette & Luckhurst,

67   1999). The annual harvest of baitfishes in Bermuda peaked at 105,072 kg in 1988, corresponding

68   with the peak of local trap fishing activity, and fell to a low of 26,842 kg in 1995 (Butler et al.,

69   1993; Smith-Vaniz, Collette & Luckhurst, 1999). Long-term landings statistics show that, after a

70   decade of adjustment, total reported commercial catches of baitfish species have remained

71   largely stable in the range of 30,000 - 40,000 kg per annum between 1999 and 2017 (Bermuda

72   Government Department of Environment and Natural Resources, pers. comm.,

73   www.environment.bm). The Dwarf herring and Bermuda anchovy, which, along with the Reef

74   silverside are collectively called 'fry', make up the bulk of the baitfish harvest (Smith-Vaniz,

75   Collette & Luckhurst, 1999). However, anecdotal evidence from fishers, together with landings

76   records for the larger baitfish species, suggests that local populations of at least some species

77   have declined over the past 40 years.

78   For species targeted by fishing, overexploitation can lead to population declines (Ecoutin

79   et al., 2010; Last et al., 2010; Stagličić et al., 2011). Other anthropogenic impacts in coastal

80   areas, such as pollution and habitat degradation (Kennish, 2002; Hewitt et al., 2008; Johnston &

81   Roberts, 2009), can also alter fish distribution patterns, decreasing richness and abundance

82   across various spatial and temporal scales (Sax & Gaines, 2003; Johnston & Roberts, 2009).

83   Apparent declines in the abundance of baitfishes in Bermuda may therefore reflect natural

84   fluctuations in the abundance and distribution of these species, or may be indicative of

85   overfishing or other anthropogenic impacts. However, a significant change in fishing practices,

86   such as the banning of fish traps, may also affect the ways in which a related resource, such as

87   bait, is harvested and used, which may in turn affect how that resource is perceived and

88   monitored by fishers.

89   At present, regulation of baitfishing in Bermuda under the Fisheries Act, 1972, and the

90   Fisheries Regulations, 2010, includes both gear and spatial restrictions. In particular, there are

91   four inshore bays (Somerset Long Bay, Shelly Bay, Whalebone Bay, and Coot Pond), within

92   which the use of fishing nets and the removal of baitfishes is completely prohibited (Bermuda

93   Fisheries Act, 1972, Section 8i), but these bays are not evenly distributed around the island (Fig.

94   1). Further, given the similarities in species morphologies, visual identification of baitfish

95   species in the field can be difficult, and a higher diversity may exist within baitfish schools than

96   is currently perceived. Thus, increased understanding of how assemblage composition varies

97   across locations, along with the extent of local movements and genetic mixing, is required to

98   inform management.

99   Lastly, if Bermuda's baitfish populations are indeed experiencing declines, a greater

100   understanding of regional genetic connectivity could indicate whether or not larval supply from

101   other populations might assist with their recovery (see Cowen & Sponaugle, 2009). Bermuda's

102   isolated, mid-Atlantic location (Fig. 1, inset) reduces the likelihood of regular supply of larvae

103   from external sources (Schultz & Cowen, 1994), and there is evidence indicating that at least

104   some local fish populations are self-seeding (see Locke et al., 2013). However, despite this

105   isolation, Bermuda was the first location outside of the eastern coast of the United States where

106   invasive lionfish were detected (Whitfield et al., 2002), indicating that larval transport and / or

107   post-larval rafting in association with floating material across this distance is not only possible

108   but occurs at a rate that enabled successful establishment of an invasive species (Locke et al.,

109   2013). Further, Bermuda has low rates of endemism (Smith-Vaniz, Collette & Luckhurst, 1999),

110   suggesting at least some genetic connectivity with other regions (see Locke et al., 2013).

111   Using genetic barcoding of the Cytochrome c oxidase 1 gene (COI), we aim to confirm

112   species identity, assess intraspecific genetic diversity, and determine rates of local and regional

113   genetic connectivity of Bermuda's baitfish populations. Barcoding has proven useful for species

114   verification in families with a high degree of morphological similarity, and has been able to

115   identify new species by integrative taxonomic analysis (Ward, Hanner & Hebert, 2009).

116   Furthermore, population genetic analyses can suggest points of origin in mixed populations and

117   provide insights to breeding structures (Allendorf & Utter, 1979). Thus, results of this study will

118   provide insights into population stability and can be used to inform future management

119   strategies.

120

121   **Methods**

122   Samples were collected from 10 locations around the islands of Bermuda: the Bermuda

123   Aquarium Museum and Zoo dock in Flatts (BAMZ), Bailey's Bay, Coney Island, Whalebone

124   Bay, Turtle Bay, South Bay, East Whale Bay, West Whale Bay, Frank's Bay, and Deep Bay,

125   between July and August 2017 (Fig. 1 – clockwise from center). All samples were collected with

126   permission of the Bermuda Government Department of Environment and Natural Resources

127   under special permit SP170303. A total of 111 individuals were collected and visually identified

128   in the field based on previously described gross morphological characteristics (Smith-Vaniz,

129   Collette & Luckhurst, 1999). Based on these initial examinations, five species were identified:

130   *Hypoatherina harringtonensis* (Reef silverside), *Anchoa choerostoma* (Bermuda anchovy),

131   *Jenkinsia lamprotaenia* (Dwarf herring), *Harengula humeralis* (Redear herring) and *Sardinella*

132   *aurita* (Round sardinella). Representative samples of each species from each location were

133   preserved in 95% ethanol for subsequent genetic analyses.

134   Genomic DNA was extracted from muscle tissue of samples using a Qiagen DNA Blood

135   and Tissue extraction kit following the manufacturer's protocols, resulting in a final volume of

136   200 μl. The cytochrome c oxidase (COI) gene was amplified from extracted DNA using a primer

137   cocktail developed for fish barcoding as described by Ivanova et al. (2007) (COI-3: C_FishF1t1-

138   C_FishR1t1). All PCRs had a total volume of 12.5 μl and included: 6.25 μl of 5% DMSO, 2.00

139   μl of H_2O, 1.25 μl of 10x Buffer [10mM KCl, 10mM $(NH_4)SO_4$, 20 mM Tris-HCl (pH 8.8),

140   2mM $MgSO_4$, 0.1% Triton X-100], 0.625 μL $MgCl_2$ (50mM), 0.125 μl of each primer cocktail,

141   0.0625 μl of DNTP (10 mM), 0.0625 μl of *Taq* Polymerase (Invitrogen), and 2 μl of DNA

142   template. PCR was optimized at the following: 95°C for 2 minutes, 35 cycles of 94 °C for 30

143   seconds, 52°C for 30 seconds, 72°C for 1 minute, with a final extension at 72°C for 10 minutes.

144   PCR products were visualized using a 1.2% agarose gel, with concentration and purity measured

145   using a spectrophotometer. Products were bi-directionally sequenced with universal M13 primers

146   using Sanger Sequencing services provided by Gene Codes Corporation. Resulting sequences

147   were manually edited and aligned using Sequencher[®] 5.4.6 (Gene Codes Corporation, Ann

148   Arbor, MI, USA) and compared to known sequences in NCBI Blast and GenBank. Accession

149   numbers are listed in Appendix 1, all sequences are available on GenBank

150   (www.ncbi.nlm.nih.gov/Genbank). Available sequences of the COI gene for conspecifics from

151   locations outside Bermuda were downloaded from GenBank and used for regional comparisons

152   of genetic structure and connectivity (Valdez-Moreno et al., 2010; Lavoué, Konstantinidis &

153 Chen, 2014). Sequences from *Anchoa lamprotaenia* [Hildebrand, 1943] from Florida (Weigt et

154 al., 2012; Lavoué, Konstantinidis & Chen, 2014) were used for comparisons with *A.*

155 *choerostoma*, as no COI sequences were available for *A. choerostoma*.

156   Sequences for all species, including those obtained from GenBank, were aligned using

157 MUSCLE (Edgar, 2004). Single gene phylogenetic analysis of the genera examined was

158 conducted using the Maximum Likelihood method based on the Tamura-Nei model (Tamura &

159 Nei, 1993). Initial trees for the heuristic search were obtained automatically by applying

160 Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the

161 Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior

162 log likelihood value. The analysis involved 154 nucleotide sequences, with a total of 718

163 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al.,

164 2018).

165   Diversity was assessed within and among locations in Bermuda using standard diversity

166 indices, including number of haplotypes (*Nh*), number of polymorphic sites (*Np*), haplotypic

167 diversity (*h*) (Nei, 1987), nucleotide diversity (*pn*) (Tajima, 1983; Nei, 1987), and mean number

168 of pairwise differences (*pd*) between haplotypes (Tajima, 1983) calculated for each species using

169 DnaSP v.5 (Librado & Rozas, 2009). Population (location) pairwise F_{ST} (Hudson, Slatkin &

170 Maddison, 1992) values, whose significances were assessed through 10,000 permutation tests,

171 were used to calculate differentiation between locations within Bermuda, as well between

172 Bermuda and other regional locations, using ARLEQUIN version 3.5.2 (Excoffier & Lischer,

173 2010). Unweighted analysis of molecular variance (AMOVA; (Excoffier, Smouse & Quattro,

174 1992) was also performed to test hierarchical models of genetic variance using pairwise

175 differences among haplotypes as a measure of divergence within and among locations in

176   Bermuda as well as within and among regional locations using ARLEQUIN version 3.5.2

177   (Excoffier & Lischer, 2010).

178

179   **Results**

180   A total of 92 individual fish were successfully sequenced (see Appendix 1). Alignments

181   for each species were trivial and required no insertion / deletion events. Sequence identification

182   of 81 individuals confirmed initial identification, while sequence identification did not match the

183   initial field identification for 10 individuals, indicating an error rate of 11% for identification

184   based on gross morphology.

185   All three samples from Deep Bay, initially identified as *Sardinella aurita*, were

186   molecularly identified as *Opisthonema oglinum*. Five of the seven samples from the BAMZ

187   location that were initially identified as *S. aurita* were also molecularly identified as *O. oglinum*.

188   One sample from Frank's Bay and one sample from Coney Island were identified as

189   *Hypoatherina harringtonensis*, but the resulting sequences matched that of *Jenkinsia*

190   *lamprotaenia*. COI sequences of individuals morphologically identified as the endemic Bermuda

191   anchovy, *Anchoa choerostoma*, had a 99% identity match (93% query coverage) to the

192   widespread Atlantic species *A. lamprotaenia*. However, no COI sequences were available for *A.*

193   *choerostoma* on public databases for comparison.

194   The single gene tree constructed based on maximum likelihood to infer phylogenetic

195   relationships (Fig. 2) indicated that species from the genera *Harengula, Opisthonema*, and

196   *Sardinella* are more closely related to each other than to the other species examined, while

197   species from the genera *Jenkinsia* and *Anchoa* are more closely related to each other than to the

198   other species. The genus *Hypoatherina,* in the order Atheriniformes, was the most evolutionarily

199   distant from the other genera.

200   Haplotype diversity was similar among species based on overlapping standard errors

201   (Table 1). Nucleotide diversity was similar for *A. choerostoma* and *H. harringtonensis*, and for

202   *H. humeralis, J. lamprotaenia*, and *O. oglinum,* but was higher for the first two species than for

203   the latter three. The mean number of pairwise differences was highest for *A. choerostoma* at

204   3.163, decreasing to 0.934 for *J. lamprotaenia,* 0.800 for *H. humeralis,* 0.780 for *H.*

205   *harringtonensis,* and 0.429 for *O. oglinum*. Diversity of *A. choerostoma* within a given bay was

206   higher at East Whale Bay than at West Whale Bay and BAMZ (Table 1). For *H. harringtonensis,*

207   within-location diversity was higher at BAMZ and Whalebone Bay than at Frank's Bay and

208   Bailey's Bay. Diversity was similar among locations for *H. humeralis* at BAMZ and Coney

209   Island, and among all sampled locations for *J. lamprotaenia*. Likewise, diversity measures did

210   not differ among locations for *O. oglinum* from Deep Bay and BAMZ. While measures of

211   diversity were high for *S. aurita,* the low sample size (n=2) and lack of replicate sites precludes

212   their inclusion in diversity comparisons (Table 1).

213   Pairwise F_{ST} comparisons between locations within Bermuda were insignificant (α=0.05)

214   for all species examined, indicating no evidence of genetic structure and high levels of genetic

215   connectivity within and among locations (Table 2). An analysis of molecular variance used to

216   test for hierarchical population structure also indicated no significant genetic structure exists

217   among locations in Bermuda for any of the species analyzed, where the majority of variation for

218   all species was found within locations rather than among them (Table 3).

219   For all species except *H. humeralis*, pairwise F_{ST} comparisons were significant (α=0.05)

220   between populations from Bermuda and those from other regions, indicating strong evidence of

221   genetic structure and limited genetic connectivity across regions (Table 4). An analysis of

222   molecular variance, used to test for hierarchical population structure, also showed significant

223   genetic structure among regions for all species analyzed with the exception of *H. humeralis*, such

224   that the majority of variation was found among regions rather than within them (Table 5).

225

226   **Discussion**

227   Based on the COI sequences obtained from baitfish samples in this study, a higher

228   diversity of species was present within the assemblages than was initially recorded based solely

229   on morphological identification in the field at the time of sampling, with 11% of individuals

230   being misidentified. Genetic sequencing revealed the misidentification of several small

231   individuals of *Opisthonema oglinum* that had yet to develop their distinctive threadfin and were

232   thus misidentified as *Sardinella aurita*, and of two small *Jenkinsia lamprotaenia* that had been

233   schooling with *Hypoatherina harringtonensis*. As a result, the total number of species analyzed

234   increased from five to six, and included *Anchoa choerostoma*, *H. harringtonensis*, *H. humeralis*,

235   *J. lamprotaenia*, *O. oglinum,* and *S. aurita*. These results highlight the value of incorporating

236   molecular identification into assessments of species assemblages for morphologically similar

237   species, as diversity may be underestimated when based only on gross morphology (Zemlak et

238   al., 2009; Hubert et al., 2012).

239   The relationship among the limited sampling of six genera of baitfishes examined in this

240   study was analyzed using a single gene tree based on a Maximum Likelihood phylogenetic

241   approach and broadly follows established relationships (e.g. in Lavoué, Konstantinidis & Chen,

242   2014) in that the genera within the Family Clupeidae (*Harengula, Opisthonema,* and *Sardinella*)

243   were found to be more closely related to each other than to the other genera examined (Fig. 2).

244 However, while our analysis found *Jenkinsia* (Family Dussumieriidae) and *Anchoa* (Family

245 Engraulidae) to be more closely related to each other than to the species in the Clupeidae, recent

246 molecular phylogenies using multiple markers have inferred conflicting sister relationships

247 between the Dussumieriidae, the Engraulidae and the rest of the Clupeoidei (see Lavoue et al.,

248 2017 and Egan et al., 2018). Deciphering these relationships is beyond the scope of the present

249 single gene analysis of five species but, as COI sequences become available for more species,

250 these data may contribute to future analyses to further elucidate the complex evolutionary history

251 of this group of fishes. All of the aforementioned genera belong to the Order Clupeiformes,

252 while *Hypoatherina* belongs to the Order Atheriniformes. Accordingly, *H. harringtonensis* was

253 found to be the most evolutionarily distant from the other genera examined.

254   Interestingly, the COI sequence results for the endemic Bermuda Anchovy, *A.*

255 *choerostoma*, were within 1% similarity to the more broadly distributed Big-eye anchovy, *A.*

256 *lamprotaenia*. *A. choerostoma* was described by Goode in 1874 based on morphological

257 variations from congeneric species. Morphologically, it is most similar to *A. lamprotaenia, A.*

258 *januaria, A. cubana* and *A. parva* (Smith-Vaniz, Collette & Luckhurst, 1999; Nizinski &

259 Munroe, 2002). However, molecular phylogenies have found it most closely related to *A.*

260 *mitchilli* using the ITS1 region (Johnson 2003 in Smith-Vaniz & Collette, 2013), and to *A.*

261 *hepsetus* using a combination of the genes 12s, 16s, RAG1 and RAG2 (Li & Ortí, 2007), but

262 these studies did not include *A. lamprotaenia.* Of these species, *A. hepsetus* is readily

263 distinguished from the others by its longer maxilla (Nizinski & Munroe, 2002). *A. choerostoma*

264 is distinguished from *A. mitchilli* by the relative positions of the dorsal fin and anal fin (such that

265 the anal fin in *A. choerostoma* is posterior to the dorsal, whereas the origins of these fins are

266 vertically aligned in *A. mitchilli*); from *A. lamprotaenia* by having a greater number of lower gill

267   rakers (23-30 as opposed to 17-21); and from the remaining similar congenerics by having a

268   notably smaller axillary scale above the pectoral fin (Smith-Vaniz, Collette & Luckhurst, 1999;

269   Nizinski & Munroe, 2002).

270   Cytochrome c oxidase is an enzyme in the respiratory chain that catalyzes the conversion

271   of oxygen to water, a critical survival process. Encoded inside mitochondria, the Cytochrome c

272   oxidase subunit 1 gene (COI) is highly conserved among all respiring organisms and is therefore

273   not subject to selective pressures that induce mutation (Mick, Fox & Rehling, 2011). It is

274   possible, therefore, that the COI gene may not provide high enough resolution to distinguish

275   between these closely related species within the genus *Anchoa*. To further evaluate and obtain an

276   accurate assessment of endemism, multiple genes should be incorporated and compared among

277   several congeneric species. Coupled with morphological variation, detailed phylogenetic

278   analyses could provide further insights into the classification of the Bermuda anchovy.

279   Of the locations examined around Bermuda, BAMZ had the highest species diversity,

280   with all six species found at this location (Fig. 1). Conversely, at several locations, only a single

281   species was found. However, this may reflect sampling effort rather than actual diversity, as

282   these sites were sampled less frequently than BAMZ. Yet, BAMZ is also the most centrally

283   located site on the more protected northern shore of Bermuda, and may, therefore, represent an

284   area of species accumulation (Tittensor et al., 2010), at least for inshore species, that results in

285   higher baitfish diversity. Among the species, *J. lamprotaenia* was the most widely distributed,

286   being found at five of the 10 locations, followed by *H. harringtonensis* at four of the 10

287   locations. Given the high estimates of connectivity among locations for all six species examined,

288   however, distributions are likely wider than reflected by the somewhat limited sampling effort in

289   this study.

290   Estimates of genetic diversity within and among baitfish assemblages in Bermuda

291   indicate high degrees of mixing between locations for all six species examined. For these small-

292   bodied species, this mixing likely occurs predominantly during the larval phase (Schultz, 2000;

293   Lavoué, Konstantinidis & Chen, 2014), but may also occur during later life stages as a result of

294   short- or long-term movements between locations that may be driven by food availability,

295   predator density, reproductive cycles or adverse conditions (Hugie & Dill, 1994; Olsson et al.,

296   2006; Udyawer et al., 2013; Currey et al., 2015). These high rates of local connectivity mean that

297   management should consider Bermuda's baitfish species as single, highly mixed populations. As

298   such, the distribution of bays that are closed to net fishing in order to protect them is of less

299   importance than it might be if subregional genetic differentiation had been detected, and there is

300   no immediate need to close additional bays in central or western parishes, or along the south

301   shore, in order to maintain local genetic diversity.

302   On a larger scale, most marine species occurring in Bermuda are restricted to the Tropical

303   Northwestern Atlantic biogeographic province (Spalding et al., 2007; Locke et al., 2013), which

304   is largely equivalent to the Caribbean biogeographic province of Kulbicki et al. (2013), and the

305   baitfish species examined here generally follow this distributional pattern. In contrast, *S. aurita*

306   is found on both sides of the Atlantic Ocean and into the Mediterranean Sea (Aquamaps, 2016).

307   However, range size does not necessarily reflect modern dispersal patterns (Lester et al., 2007)

308   and, for marine fishes, small body size is correlated with lower dispersal capacity and increased

309   rates of endemism (DeMartini and Friedlander 2004; Bradbury et al 2008). This seems likely to

310   apply to baitfish species, with implications for regional connectivity for an isolated island like

311   Bermuda.

312   The present study documents significant genetic differentiation between conspecific

313   populations of baitfishes in Bermuda and those in other regions (Table 4; F_{ST}; p<0.05), and

314   significant divergence among regions (Table 5; AMOVA; p<0.05) for *J. lamprotaenia*, *H.*

315   *harringtonensis*, *O. oglinum,* and *S. aurita,* as well as between congeneric populations of *Anchoa*

316   *sp.* among regions. These results suggest that gene flow, and therefore exchange of individuals,

317   is limited between populations of these species in Bermuda and those in other regions.

318   Populations of *H. humeralis*, on the other hand, show no evidence of genetic structure,

319   suggesting broad genetic connectivity exists across the Caribbean / Tropical West Atlantic for

320   this species. Importantly, these results have major implications for management as strong genetic

321   divergence in *J. lamprotaenia*, *H. harringtonensis*, *O. oglinum,* and *S. aurita* suggests that

322   populations of these species in Bermuda are likely self-seeding and locally maintained. Thus,

323   local declines in baitfish abundances are not likely to be replenished from distant populations.

324

Conclusion

326   Baitfishes depend on shallow inshore areas, which are capable of sustaining great

327   diversity and densities of organisms (Nagelkerken et al., 2001; Ray & Carleton Ray, 2005;

328   Vasconcelos et al., 2011; Araújo et al., 2017), but are also extensively modified and threatened

329   by human activities such as overfishing, pollution, coastal development and habitat degradation,

330   which may impact fish communities (Kennish, 2002; Sax & Gaines, 2003; Ribeiro et al., 2008;

331   Johnston & Roberts, 2009; Ecoutin et al., 2010; Last et al., 2010; Araújo et al., 2017). The

332   limited genetic connectivity of baitfish populations among Western Atlantic regions documented

333   here indicates restricted influx of new individuals to Bermuda and highlights the vulnerability of

334   local populations to natural and anthropogenic perturbations. As such, it is important to monitor

335   both fish communities and environmental parameters in Bermuda's nearshore habitats (Araújo et

336   al., 2017), and to adapt management measures accordingly, in order to conserve these

337   ecologically and economically important species.

338

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342

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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

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			

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		

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

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		

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.
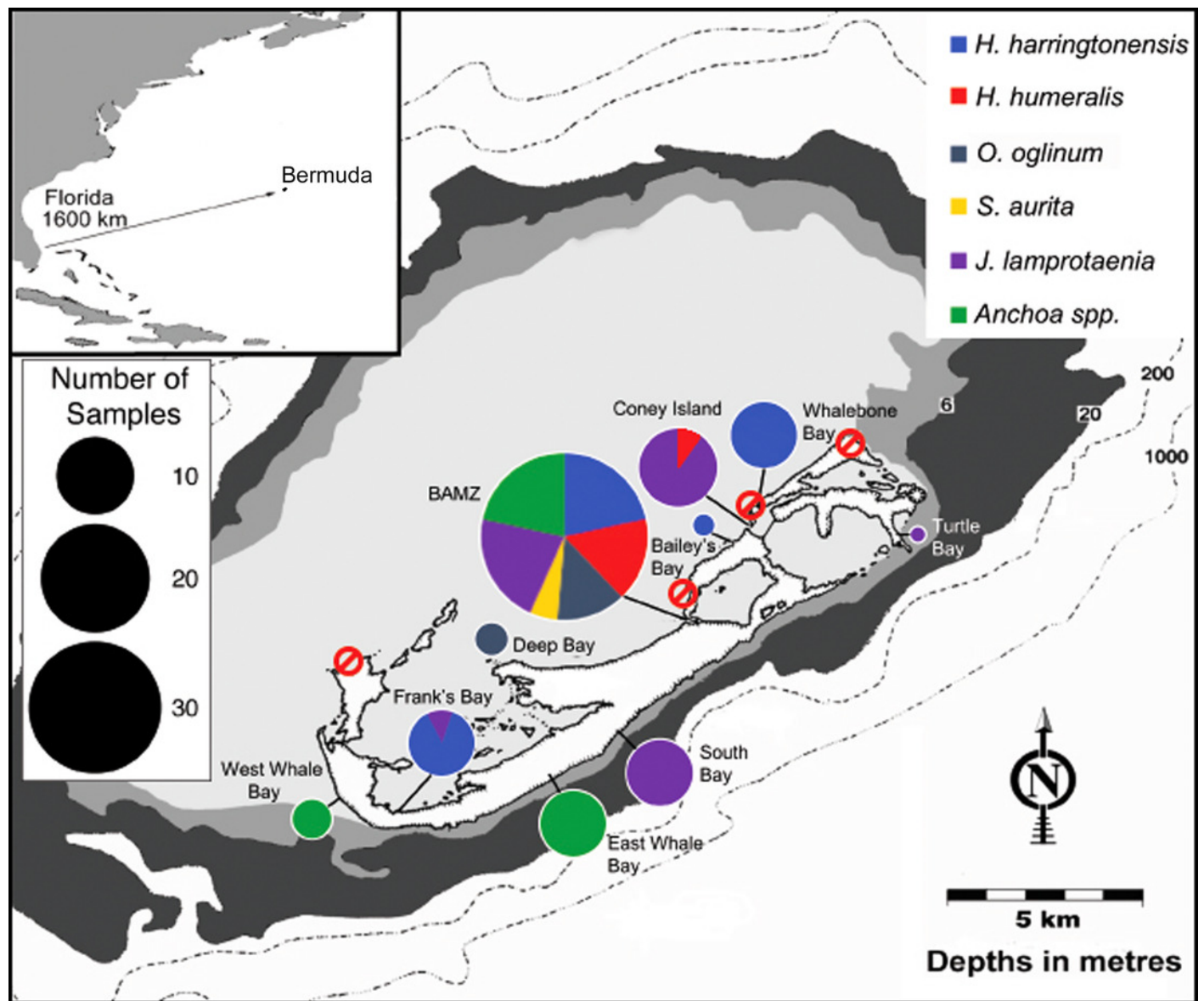
Figure 2

Molecular Phylogenetic Analysis.

The evolutionary history was inferred from a single gene tree using the Maximum Likelihood method based on the Tamura-Nei model. 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.