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Invasive lionfish dispersal between shallow- and deep-water habitats within coastal Floridian waters

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Abstract:

1. Introduced lionfish threaten native fishes and ecosystem health in Atlantic and Caribbean communities. While controlling their spread and population growth may be difficult, given their early maturity, high fecundity and a long larval dispersal period, mitigation efforts may also be limited by the existence of large and numerous, deep water refugia. Despite the potential importance of these refugia, their connection with shallow water populations and their role in recruiting pelagic larvae stays poorly understood.

2. We examined the post-settlement dispersal patterns of invasive lionfish using $\delta^{13}C$ and $\delta^{18}O$ stable isotope analysis on otoliths and Bayesian stock mixture analysis. Individuals were sampled from the Western Floridian Shelf and the Florida Keys at depths up to 105 m. The core and rim isotope compositions of otoliths were used to compare the use of shallow versus deep habitat in the early and late life stages of lionfish individuals.

3. We find that juvenile lionfish inhabit both deep and shallow waters. In the Western Floridian Shelf, the majority of lionfish likely settled into the deeper habitat between 45-90 m. There was asymmetry in larval movement with 44% of captured individuals in the shallow habitats of the Western Floridian Shelf dispersed into shallow environments post-settlement from below 45 m. However, shallow regions contributed less than 5% to habitats deeper than 45 m.

45   **4.** In contrast, individuals captured in the shallow habitat of the Keys were mostly

46   composed of shallow settlers. Around 20% of individuals from the shallow seagrass

47   beds in the Keys likely dispersed from the deeper Key sites.

48   **5.** *Synthesis and applications.* This study shows an empirical link between deep and

49   shallow water habitats. We suggest that post-settlement dispersal can contribute to the

50   invasion of new habitat and potentially hinder shallow-water removal efforts in

51   currently colonized areas. Going forward, removal efforts could target the important

52   deep-water refugia populations highlighted in this work, which will require more

53   effective strategies for controlling deep-water lionfish populations.

54   **Keywords:** Invasive Species, Lionfish, Movement, Otolith, Stable Isotopes

55   **Introduction:** Two species of lionfish (*Pterois volitans* and *P. miles*), native to the Indo-Pacific,

56   were first observed off the eastern coast of Florida in the mid-1980s (Morris and Akins 2009).

57   Lionfish exploit more than half of gape-appropriate prey species from their invaded range,

58   including fish and invertebrates, causing large reductions in total prey biomass (Green et al.,

59   2012). As a result, lionfish influence larval recruitment, alter the behavior of native fishes, and

60   contribute to the destabilization of some coral reefs (Albins and Hixon, 2008; Curtis et al., 2017;

61   Lesser and Slattery, 2011). Thus, limiting the spread of lionfish and controlling their existing

62   populations are necessary for the conservation of native communities. Larval dispersal is largely

63   responsible for the spread of lionfish throughout the Caribbean: average sized females can

64   produce on average , 2.3 million eggs per year, larvae may travel up to 26.2 days on ocean

65   currents, and extreme events like hurricanes can even carry these larvae to isolated, seemingly

66   unreachable regions (Ahrenholz and Morris, 2010; Fogg et al., 2017; Morris and Akins, 2009).

67   Currently, invasive lionfish are established from North Carolina, USA to Brazil in a

68   diverse set of habitats: including mangroves, coral reefs, seagrass beds, and deep coastal waters

69   (Barbour et al., 2011; Ferreira et al., 2015; Schofield, 2010). Lionfish are primarily restricted

70   largely by thermal constraints, allowing them to be widely successful as an exotic throughout

71   their expanding Atlantic territory (Cure et al., 2014; Kimball et al., 2004). In its invaded range,

72   lionfish tend to first colonize shallow habitats and tend to exhibit high site fidelity (Akins et al.,

73   2014; Biggs and Olden, 2011; Jud and Layman, 2012; Tamburello and Côté, 2015). As the

74   invasion process progresses, lionfish populations have been seen to appear in deeper coastal

75   regions: where populations are denser and individuals tend to be larger in size (Andradi-Brown

76   et al., 2017; Claydon et al., 2012; Gress et al., 2017; Nuttall, 2014). These studies suggest some

77   preference for shallow-water settlement in nursery habitats. Nursery habitat use is common for

78   many other reef fish that use seagrass beds and mangroves for the abundant food resources and

79   for protection from predators (Dorenbosch et al., 2005; Mumby et al., 2004; Nagelkerken et al.,

80   2000). While shallow waters are evidently a vital component of lionfish life history, deeper

81   waters may be equally important, but less well understood.

82   Often, in deeper waters, lionfish population densities are much higher than in the

83   shallower populations (Biggs and Olden, 2011; Claydon et al., 2012; Nuttall, 2014; Switzer et

84   al., 2015). Analysis of a USGS database showed lionfish occupying habitats around 35 m on

85   average and maintaining populations down to 150 m (Johnston and Purkis, 2011). Spillover from

86   deep-water refugia into shallow-waters may decrease the efficiency of any shallow-water

87   removal efforts. Missing even a small source population may lead to unsuccessful removal

88   efforts, which highlights the importance of understanding deep water contributions to shallow

89   populations (Johnston and Purkis, 2015). In fact, on the Western Floridian Shelf (WFS), models

90   predict that the invasion began further offshore, as larvae shed off the Florida Loop Current into

91   deep waters (Johnston and Purkis, 2015; Switzer et al., 2015). Recruits within the WFS could

92   either be those from the Loop current, those that move along slower currents, or those that are

93   trapped within local currents (Johnston et al., 2017). Unfortunately, control of deep-water

94   populations is more logistically more difficult than shallow-water spear fishing. However,

95   developments in lionfish-specific trapping technologies are allowing for increased deep-water

96   catches (Gittings et al., 2017, Harris et al., 2020)

97   While larval dispersal is a significant component of overall lionfish dispersal, previous

98   work indicates post-settlement dispersal as an important life history trait for other coastal marine

99   fish (Cocheret de la Morinière et al., 2002; Franco et al., 2012). Many reef fishes use nursery

100   habitats, like mangroves, before migrating offshore to reefs (de la Morinière et al. 2002). Some

101   invasive species, like rainbow trout (*Oncorhynchus mykiss*) in eastern Quebec, colonize new

102   territories along rivers through post-settlement dispersal (Thibault et al., 2010). Lionfish are not

103   typically a highly mobile species, like migrating salmonids, but do have the ability for longer

104   dispersals. Mark-recapture studies that investigate lionfish post-settlement dispersal show that

105   lionfish tend to demonstrate high site fidelity over 10-15 days (Akins et al., 2014; Jud and

106   Layman, 2012; Tamburello and Côté, 2015). In one case, 74% of individuals were recaptured

107   near their original sampling location after 10 months, indicating high site-fidelity (Jud and

108   Layman, 2012). Although in another case, individuals traversed up to 1.35 km over the course of

109   the study (Akins et al. 2014, Tamburello and Cote 2015). Recently, fine-scale acoustic tracking

110   methods have shown lionfish individuals to have larger home-ranges than expected and be

111   capable of moving ~800 m a day and 2 km over the course of 89 day (Dahl and Patterson, 2020).

112　However, both broad patterns of post-settlement dispersal, including any post-settlement

113　connections between deep and shallow waters, are not well understood, or documented.

114　　　To investigate the post-settlement dispersal patterns of invasion lionfish we use the stable

115　isotope compositions of otoliths from lionfish captured throughout Florida's coastal waters:

116　namely Marathon Key and a subset of the Western Floridian Shelf. Changes in the composition

117　of $\delta^{13}C$ and $\delta^{18}O$ along an otolith's growth axis reflect shifts in a fish's chemical environment

118　through time and can indicate dispersal events (Pracheil et al., 2019). Stable isotope analysis on

119　otoliths is commonly used to describe settlement, dispersal, and connectivity within marine fish

120　populations (Bouchoucha et al., 2018; Campana and Thorrold, 2001; Kitchens et al., 2018; Reis-

121　Santos et al., 2015; Weidman and Millner, 2000). Post-settlement dispersion can be identified

122　through differences between core and rim isotope compositions and stock mixture analysis.

123　Environments along the inshore – offshore gradient, that correlates to trends in $\delta^{13}C$ and $\delta^{18}O$

124　isotopes, should be more different from eachother than those that fall within chemical isoclines

125　across similar depths (Cocheret de la Morinière et al., 2002; Kalish, 1991; Michener and Lajtha,

126　2008; Paillon et al., 2014). Thus, individuals that disperse further along the inshore and offshore

127　gradient away from their settlement grounds are expected to have larger differences between rim

128　and core isotopes. Specifically, this study tests: 1) if individuals tend to settle in deeper or

129　shallower habitat; and 2) if post-settlement dispersal connects deep and shallow water habitats.

130　Given the earlier work, indicating high site fidelity, we expect to see most individuals have

131　similar natal and capture habitats. As densities of juveniles are often higher in shallow habitat

132　and there are indications of potential ontogenetic shifts towards deeper habitat, we expect that a

133　fraction of otoliths from individuals captured in deep water habitats will reflect use of shallow,

134　warmer natal habitats and subsequent offshore dispersals.

Methods:

Sampling: We sampled sagittal otoliths from 32 adult lionfish from Marathon Key and the Western Floridian Shelf (WFS) (Fig. 1). Otoliths from the Florida Keys were sampled from carcasses that were byproducts of recreational fishers and dive shops. As the majority of these carcasses were cleaned and filleted by the anglers, data on fish length and weight was not always available, but approximate depth and location was available for all analyzed individuals. All otoliths sampled from the WFS were subset from a collection of otoliths that had been previously acquired for the comparison of lionfish age and growth across the Gulf of Mexico (Fogg et al., 2019). These individuals were collected opportunistically by spear fishers and trawlers (both commercial and fisheries-independent) (for additional information on specimen collection, see Fogg et al., 2017). Otoliths in the WFS were sampled down to 105 m and were binned into 45 m depth steps: 0 - 45 m, 45 - 90 m, > 90 m. Each fish from WFS had associated metadata of depth at capture, approximate GPS points, and a habitat type. Otoliths sampled from the Florida Keys were binned to separate samples from the shallow seagrass flats along the inner coast of Marathon Key (0 - 10 m) and the shipwreck sites along the Atlantic coast of the Florida Keys (> 10 m).

We sampled the core and the rim of the otolith, using a micro-mill, to assess the chemical environment of each fish at two time points: this included early otolith (juvenile) growth and most recent otolith (adult) growth. Otoliths were cleaned using a Sonic: vibra cell. Each otolith was sonicated for 6 – 7 seconds at 60% amplitude to remove contaminants. Samples were dried and mounted to glass slides with superglue for the micro-mill process. The drill and working area were cleaned between samples to avoid contamination. Between 50-80 micrograms of otolith material were analyzed for $\delta^{13}C$ and $\delta^{18}O$ at the Lamont-Doherty Earth Observatory on a

158   Thermo-Fisher Delta V+ mass spectrometer with dual-inlet and Kiel IV carbonate reaction

159   device. Weighed sample powders were dissolved in ~100% H_3PO_4 at ~70°C. NBS-19 standards

160   were analyzed 5–6 times per day. To assess external precision and sample homogeneity replicate

161   core and rim samples were run for an individual (N = 3) (Table 1). Internal NBS-19 standards

162   were run every 10th sample across the entire run (Table 1).

163   Statistical Analysis: An ANOVA was used to assess differences in otolith isotope

164   compositions between depth zones. The isotopic compositions of WFS otoliths were analyzed

165   with Bayesian stock mixture in the R package 'mix.Fish' (R version: 3.5.1). Stock mixture

166   analysis is a means of analyzing the unknown stock composition of a mixed stock using know

167   traits, such as genotypes or isotope compositions, from known stocks that could contribute to the

168   mixed stock (Pella and Masuda, 2001). Known stocks, or base stocks, are characterized through

169   otolith rim observations, as their exact location of capture is known, and it is assumed that the

170   individuals are residents in that location (Brophy et al., 2016; Pella and Masuda, 2001; Smith and

171   Campana, 2010). Core isotope compositions, reflections of juvenile habitat use, are not directly

172   observed through capture. This analysis is aimed at estimating where adults at each depth step

173   originated from by considering the core observations as the mixed stock sample and determining

174   the contribution of each depth to that mixed sample. The analysis uses WinBUGS software

175   (Bayesian inference Using Gibbs Sampling, (Spiegelhalter et al., 2001) through the R package

176   R2WinBUGS and MCMC methods. DIC, a metric of expected predictive error, is calculated as

177   the posterior mean of deviance plus the estimated effective number of parameters found in the

178   posterior distribution (Spiegelhalter et al. 2001). The Bayesian stock mixture model was run for

179   50K iterations across 5 chains with the first 25K discarded as burn-in.

180   **Results:** The isotopic compositions of otoliths in this study differed significantly across depth

181   within the WFS for $\delta^{18}O$ ($F(1, 33) = 23.95$, $p < 0.001$) and for $\delta^{13}C$ ($F(1, 33) = 6.857$, $p = 0.013$)

182   . $\delta^{18}O$ increased with depth ($R^2 = 0.42$, $F(1,33) = 23.95$, $p < 0.001$) and $\delta^{13}C$ decreased with

183   increasing depth ($R^2 = 0.17$, $F(1,33) = 8.857$, $p = 0.013$) (Fig. 2). All chains converged as

184   indicated by a scale reduction factor (Rhat) of close to 1. The DIC diagnostic of expected

185   predictive error is 257.1.

186   The sampled WFS region between 45 – 90 m is estimated to be the region with the

187   highest overall settlement given how frequently this depth region was predicted as the location of

188   otolith core development. The individuals predicted to settle between 45 – 90 m were captured

189   across all sampled regions in the WFS. For example, 45 – 90 m settlers composed 36% of the

190   WFS population < 45 m, acting as the largest source of individuals to the shallow (< 45 m) WFS

191   habitat. The individuals that settled between 45 – 90 m composed ~ 85% of the sampled

192   population below 90 m. Additionally, ~ 87% of the individuals from the 45 – 90 m region are

193   estimated to have settled there, indicating a high rate of settler retention given the lack of a long-

194   term change in otolith isotope composition. Interestingly, this is the highest rate of settler

195   retention within this study. We estimated that both the shallow and the deepest (> 90m) WFS

196   sites had low levels of juvenile retention: ~ 7% of the population and ~ 4% of the population

197   respectively (Fig. 3; Table 2).

198   Larvae settling above 45 m in the WFS were estimated to contribute only a small

199   proportion of individuals to deeper WFS sites: ~ 2% of the 45 – 90 m population and ~ 2% of the

200   > 90 m populations. Combined with their low rates of settler retention, the settlers of < 45 m

201   make up only a small proportion of the broader WFS community. Similarly, settlers of the

202   regions > 90 m deep contribute minimally to other sampled regions: ~ 2% of the 45 – 90 m and ~

203   8% of the < 45 m populations. Overall, natal habitat between 45 m – 90 m appears to be

204   disproportionally important to all other sampled WFS depth regions as a source of settlers. The

205   general flow of individuals radiates both inshore and offshore from this zone. While there are

206   smaller exchanges of individuals between all WFS depths, and some occurrence of settler

207   retention, these flows are minimal (Fig. 3; Table 2).

208   Within the shallow seagrass beds, < 10 m, along the Northern side of Marathon Key,

209   many of the individuals (~64%) were larval recruits. Some 20% of individuals likely used natal

210   habitat in the deeper waters along the Atlantic side of the Keys. Interestingly, the model

211   estimated that the remaining proportion of individuals within the < 10 m habitat along Marathon

212   Key were sourced from the WFS, indicating exchange between the two regions. The shallow

213   WFS (>45 m) contributed ~ 10% of individuals captured in the Marathon seagrass flats. The

214   deeper areas of the WFS, 45-90 m and > 90 m both contributed ~ 3% of the estimated population

215   in the seagrass flats. The exchange was not unidirectional but was asymmetrical as both sampled

216   regions in the Keys also contributed individuals to the WFS. The seagrass beds of Marathon

217   contributed ~ 30% of individuals to shallower WFS sites < 10 m. Deeper sites off the Keys

218   contributed ~ 18% of the population in shallow WFS sites. These contributions from the Keys

219   make the shallower WFS region a nearly 50/50 mixture of Keys and WFS individuals. The

220   deeper region of the Keys contributed ~ 7% of sampled adult populations at both deeper WFS

221   sites: 45 – 90 m and > 90 m (Fig. 3; Table 2).

222   **Discussion:** Overall, our stable isotope analysis of lionfish otoliths empirically demonstrates the

223   use of deep-water habitat by juveniles and indicates that many individuals disperse from their

224   juvenile habitat. Invasive lionfish colonize a diverse set of habitats throughout their invaded

225   range (Barbour et al., 2011; Claydon et al., 2012), with notably high numbers throughout deeper,

226 offshore waters (Biggs and Olden, 2011; Claydon et al., 2012). Previous studies have suggested

227 that new invasions of lionfish begin in shallow waters (Biggs and Olden, 2011; Gress et al.,

228 2017) and that densities of adults are higher in deep-water refugia when compared to nearby

229 shallower habitats. Here we assess: 1) if individuals tend to settle into deeper or shallower

230 habitat; 2) if post-settlement dispersal connects deep and shallow water habitats. Past

231 observational studies have emphasized the potential importance of shallow water habitat for

232 juvenile lionfish (Andradi-Brown et al., 2017; Biggs and Olden, 2011). However, our study

233 highlights the importance of both shallow and deep habitats and the post-settlement dispersal

234 across those depths.

235 Juvenile lionfish often use nursery habitats, occurring there in high densities (Andradi-

236 Brown et al., 2017; Biggs and Olden, 2011). However, in areas along the WFS, juveniles and

237 adults utilize the same habitats in the absence of established nursery grounds in close proximity

238 (Dahl et al., 2018; Stevens et al., 2006). We demonstrate juvenile lionfish habitat use across a

239 depth gradient: with hotpots in shallow, nursery habitat (e.g., Marathon Key) and deep, offshore

240 environments (e.g., WFS). A majority of the WFS lionfish individuals use natal habitat between

241 depths of 45 – 90 m, emphasizing the importance of deep environments. Overall, settlement

242 within this region composed around 53% of total modelled natal habitat. Marathon key hosts

243 another region of high settlement, but one that composed a smaller percent of overall settlement

244 and that emphasized the importance of shallow nursery habitat.

245 Our results demonstrate that most lionfish are not lifelong residents of a certain depth.

246 While deeper waters have been cited as important refugia for adult lionfish, these findings

247 indicate that deep water habitats may also be important for lionfish recruitment. Across depths,

248 40% of individuals showed no evidence of having dispersed away from their natal environment.

249   This pattern could result from site fidelity or smaller dispersals that are undetectable with our

250   methods. Lionfish are slow-moving, demersal, predators that tend to exhibit high site fidelity

251   (Akins et al., 2014; Green et al., 2019; Jud and Layman, 2012; Morris Jr and Whitfield, 2009;

252   Tamburello and Côté, 2015), but our results suggest that more than half of individuals move far

253   enough throughout their lifetime to demonstrate differences in isotopic signature. Half of the

254   individuals exhibiting habitat fidelity are modelled to reside between 45 – 90 m, where there is

255   the highest frequency of recruit retention and habitat fidelity in our study. Shallow water habitats

256   are also important for settlement and retention: ~ 40% of the other individuals who remained

257   within-habitat over the course of their lifetime resided at Marathon Key.

258   Our isotopic analysis connects deep and shallow lionfish populations by tracking

259   dispersals away from these important juvenile habitats. Fish may disperse as individuals outgrow

260   predation pressures or develop new dietary (Laegdsgaard and Johnson, 2001). In other cases,

261   dispersal can be density dependent or a product of spillover (Abesamis and Russ, 2005; Travis et

262   al., 1999). Spillover from high density populations occurs in both natural habitats and managed

263   areas, resulting in an increased population of individuals within nearby areas (Blitzer et al., 2012;

264   Casini et al., 2012; Tscharntke et al., 2005). Spillover from high-density regions, could explain

265   their widespread contributions to other depth zones as individuals search for less competitive

266   environments and better food resources. In areas of high density, invasive lionfish grow at slower

267   rates, with individual growth declining linearly with population density (Benkwitt, 2013).

268   Furthermore, lionfish adults tend to demonstrate decreased site fidelity under high density

269   pressures and may seek out low-density habitat across distances as large as 300 m (Dahl et al.,

270   2016; Tamburello and Côté, 2015). However, in other high-density experiments, there was no

271   evidence of density-dependent trends in recruitment, immigration, or loss (Benkwitt, 2013).

272 However, higher incidences of cannibalism have been recorded, indicating larger individuals are

273 consuming smaller lionfish within high density populations (Dahl et al., 2018). High population

274 density within the 45 – 90 m region may explain the high contribution of this depth zone to those

275 adjacent to it, but without data on population density at these sites, we are unable to determine if

276 this is the case.

277 The Floridian Loop Current may be the driving force behind the settlement and dispersal patterns

278 observed in this study. The Loop Current is a fast-moving current that transports water through

279 the Gulf of Mexico and around Florida (Fig. 4). It has been implicated, in theoretical work, as a

280 potential source of lionfish larvae to the Gulf of Mexico and WFS, allowing for early

281 colonization of the area through deep water colonies that move shoreward (Johnston and Purkis,

282 2015; Switzer et al., 2015). High density populations in the offshore WFS are predicted sources

283 while lower density sinks are predicted to persist inshore (Johnston et al., 2017). Along the

284 continental shelf, currents are slower and more variable in direction, possibly allowing lionfish

285 individuals to radiate throughout the region (Johnston and Purkis, 2015; Switzer et al., 2015).

286 Lionfish recruits could be those that shed off faster moving currents, moved along slower

287 currents, or were self-recruits trapped in areas of slow-moving current slower moving currents

288 would also allow a flow from offshore to inshore environments, even without impressive

289 swimming capabilities. The loop current runs close enough to our sampled sites that we

290 conjecture this is the source of many larvae in the region. Our observed high settlement within

291 the 45 – 90 m depth zone may be a result of larval lionfish falling off of the loop current. Our

292 findings provide the first empirical support for this theoretical work through the novel use of

293 otolith stable isotopes in this system.

294   Our model suggests some WFS settlement outside of the 45 – 90 m habitat and

295   subsequent dispersion away from those regions. While this estimated proportion of settlement is

296   smaller in magnitude, it demonstrates that larvae are settling across a large range of depths.

297   Coastal recruitment, implicated to be a major driver of the lionfish invasion throughout other

298   Caribbean systems, plays a surprisingly low role in contributing individuals throughout other

299   sampled WFS regions. This may be due to current pathways assisting shallower settlers to other,

300   unsampled regions of the WFS. Alternatively, those coastal regions may produce fewer juvenile

301   lionfish. Another potential explanation could involve density dependent factors. If settlement is

302   higher between 45 – 90 m, it may be harder for individuals to successfully move into that high

303   density environment. If they do succeed, there may be a sampling bias towards the more

304   abundant local recruits. Lower densities along the coast may be due to less frequent settlement or

305   potentially higher pressures from spear fishers. Either way, lower population densities of lionfish

306   would reduce spillover to surrounding regions and may also explain observed patterns.

307   We see more exchange between the shallow WFS region and the shallow Keys region

308   than we see the coastal WFS contributing to other WFS regions. Around 30% of individuals

309   captured in the coastal WFS habitat are estimated to have settled first in habitat more similar to

310   Marathon Key. The Atlantic coast of the Keys is estimated to contribute 18% of the coastal

311   WFS. Additionally, the decreasing contributions from further away regions could be reflective of

312   increasing environmental or biological filters between these regions. A much smaller proportion

313   of individuals disperse into deeper regions of the WFS. However, it appears that ~10% of

314   individuals captured in the Keys may have come from habitat more similar to the coastal WFS

315   habitat. While our model indicates that the majority of post-settlement lionfish dispersal occurs

316   in the direction of the average current flow, this lionfish dispersal towards the Keys suggests that

317 the average direction of current flow may not be the only factor that influences the direction

318 post-settlement dispersal.

319 **Conclusions:** Overall, these observed patterns indicate that lionfish dispersal is complex

320 phenomenon, but certain depth zones appear to be key recruitment bands. It can be aided by

321 currents but may occur opposite the average flow directions. There is settlement in both cooler,

322 deep, and warmer, shallow habitats. These settlement zones contribute, at least to a small extent,

323 to all other regions sampled. Focusing removal or conservation effort on source populations is a

324 well-known method of controlling the growth of a target species (Conner and Morris, 2015;

325 Lepak et al., 2006; Weidel et al., 2007). As removal of lionfish from deeper coastal water is more

326 difficult than shallow water removal, the existence of deep areas of natal importance may hinder

327 lionfish population control. While there does appear to be connectivity between all sampled

328 regions, the dispersal of lionfish can be generalized as inshore and offshore movement of lionfish

329 within the WFS away from 45-90m and movement along current flows from the Atlantic Key

330 shores towards the WFS. Lastly, there is evidence of long-term habitat specificity and settler

331 retention in both 45 – 90 m WFS and Keys > 10 m. Our localized data suggest that post-

332 settlement dispersal may be an overlooked component of lionfish invasion biology. Observed

333 populations have high connections to particular regions. However, our study area reflects only a

334 subset of usable habitat within Floridian coastal waters, so we are unable to speak to broader

335 spatial and temporal patterns within this system. Populations may grow, self-recruit, or radiate

336 into new regions due to spillover or opportunistic use of ocean currents. Further work should be

337 directed towards understanding if larval settlement is passive or deliberate in search of a certain

338 habitat: potentially a preference for a certain terrain or rugosity. Increase sampling of areas

339 throughout the entire study region could aid in identifying other settlement locations. While our

340 results are specific to smaller regions within a broader landscape, this study may inform broader

341 trends concerning the role of deep-water refugia throughout the invaded western Atlantic and the

342 role of currents as enablers of post-settlement dispersal.

350 **Data Statement**

351 The data for this study are available via the Zenodo digital repository:

352 (https://doi.org/10.5281/zenodo.7015879)

353 The Group for High Resolution Sea Surface Temperature (GHRSST) Multi-scale Ultra-high

354 Resolution (MUR) SST data were obtained from the NASA EOSDIS Physical Oceanography

355 Distributed Active Archive Center (PO.DAAC) at the Jet Propulsion Laboratory, Pasadena, CA

356 (http://dx.doi.org/10.5067/GHGMR-4FJ01).

357 **Conflict of Interest**

358 The authors have no conflicts of interest to declare

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361 **Author Contributions**

362 *MEA and JAD conceived the ideas and designed methodology; MEA and AQF collected the*

363 *data; MEA analyzed the data; MEA led the writing of the manuscript. All authors contributed*

364 *critically to the drafts and gave final approval for publication.*

365 *Statement of Inclusion*: Our study was conducted in the USA, the home-country of all authors.

366 Local scientists and stakeholders we're included in the data collection component of the study.

367 One of the contributing authors is a researcher local to Florida and efforts were made to include

368 literature from Florida. However, more could have been done to engage with local researchers

369 and distribute the results to stakeholders.

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383   Bibliography

384   Abesamis, R.A., Russ, G.R., 2005. Density-Dependent Spillover from a Marine Reserve: Long-Term
385   Evidence. Ecol. Appl. 15, 1798–1812. https://doi.org/10.1890/05-0174
386   Ahrenholz, D.W., Morris, J.A., 2010. Larval duration of the lionfish, <Emphasis Type="Italic">Pterois
387   volitans</Emphasis> along the Bahamian Archipelago. Environ. Biol. Fishes 88, 305–309.
388   https://doi.org/10.1007/s10641-010-9647-4
389   Akins, J.L., Morris, J.A., Green, S.J., 2014. In situ tagging technique for fishes provides insight into growth
390   and movement of invasive lionfish. Ecol. Evol. 4, 3768–3777. https://doi.org/10.1002/ece3.1171
391   Albins, M., Hixon, M., 2008. Invasive Indo-Pacific lionfish Pterois volitans reduce recruitment of Atlantic
392   coral-reef fishes. Mar. Ecol. Prog. Ser. 367, 233–238. https://doi.org/10.3354/meps07620
393   Andradi-Brown, D.A., Vermeij, M.J.A., Slattery, M., Lesser, M., Bejarano, I., Appeldoorn, R., Goodbody-
394   Gringley, G., Chequer, A.D., Pitt, J.M., Eddy, C., Smith, S.R., Brokovich, E., Pinheiro, H.T., Jessup,
395   M.E., Shepherd, B., Rocha, L.A., Curtis-Quick, J., Eyal, G., Noyes, T.J., Rogers, A.D., Exton, D.A.,
396   2017. Large-scale invasion of western Atlantic mesophotic reefs by lionfish potentially
397   undermines culling-based management. Biol. Invasions 19, 939–954.
398   https://doi.org/10.1007/s10530-016-1358-0
399   Barbour, A.B., Allen, M.S., Frazer, T.K., Sherman, K.D., 2011. Evaluating the Potential Efficacy of Invasive
400   Lionfish (Pterois volitans) Removals. PLOS ONE 6, e19666.
401   https://doi.org/10.1371/journal.pone.0019666
402   Benkwitt, C.E., 2013. Density-Dependent Growth in Invasive Lionfish (Pterois volitans). PLoS ONE 8.
403   https://doi.org/10.1371/journal.pone.0066995
404   Biggs, C.R., Olden, J.D., 2011. Multi-scale habitat occupancy of invasive lionfish (Pterois volitans) in coral
405   reef environments of Roatan, Honduras. Aquat. Invasions 6, 447–453.
406   Blitzer, E.J., Dormann, C.F., Holzschuh, A., Klein, A.-M., Rand, T.A., Tscharntke, T., 2012. Spillover of
407   functionally important organisms between managed and natural habitats. Agric. Ecosyst.
408   Environ. 146, 34–43. https://doi.org/10.1016/j.agee.2011.09.005
409   Bouchoucha, M., Pécheyran, C., Gonzalez, J.L., Lenfant, P., Darnaude, A.M., 2018. Otolith fingerprints as
410   natural tags to identify juvenile fish life in ports. Estuar. Coast. Shelf Sci. 212, 210–218.
411   https://doi.org/10.1016/j.ecss.2018.07.008
412   Brophy, D., Haynes, P., Arrizabalaga, H., Fraile, I., Fromentin, J.M., Garibaldi, F., Katavic, I., Tinti, F.,
413   Karakulak, F.S., Macías, D., Busawon, D., Hanke, A., Kimoto, A., Sakai, O., Deguara, S., Abid, N.,
414   Santos, M.N., 2016. Otolith shape variation provides a marker of stock origin for north Atlantic
415   bluefin tuna (Thunnus thynnus). Mar. Freshw. Res. 67, 1023. https://doi.org/10.1071/MF15086
416   Campana, S.E., Thorrold, S.R., 2001. Otoliths, increments, and elements: keys to a comprehensive
417   understanding of fish populations? Can. J. Fish. Aquat. Sci. 58, 30–38.
418   https://doi.org/10.1139/f00-177
419   Casini, M., Blenckner, T., Möllmann, C., Gårdmark, A., Lindegren, M., Llope, M., Kornilovs, G., Plikshs, M.,
420   Stenseth, N.C., 2012. Predator transitory spillover induces trophic cascades in ecological sinks.
421   Proc. Natl. Acad. Sci. 109, 8185–8189. https://doi.org/10.1073/pnas.1113286109
422   Claydon, J.A.B., Calosso, M.C., Traiger, S.B., 2012. Progression of invasive lionfish in seagrass, mangrove
423   and reef habitats. Mar. Ecol. Prog. Ser. 448, 119–129.
424   Cocheret de la Morinière, E., Pollux, B.J.A., Nagelkerken, I., van der Velde, G., 2002. Post-settlement Life
425   Cycle Migration Patterns and Habitat Preference of Coral Reef Fish that use Seagrass and
426   Mangrove Habitats as Nurseries. Estuar. Coast. Shelf Sci. 55, 309–321.
427   https://doi.org/10.1006/ecss.2001.0907
428   Conner, L.M., Morris, G., 2015. Impacts of Mesopredator Control on Conservation of Mesopredators and
429   Their Prey. PLOS ONE 10, e0137169. https://doi.org/10.1371/journal.pone.0137169

430   Cure, K., McIlwain, J.L., Hixon, M.A., 2014. Habitat plasticity in native Pacific red lionfish Pterois volitans
431   facilitates successful invasion of the Atlantic. Mar. Ecol. Prog. Ser. 506, 243–253.
432   https://doi.org/10.3354/meps10789
433   Curtis, J., Wall, K., Albins, M., Stallings, C., 2017. Diet shifts in a native mesopredator across a range of
434   invasive lionfish biomass. Mar. Ecol. Prog. Ser. 573, 215–228.
435   https://doi.org/10.3354/meps12164
436   Dahl, K., Patterson, W., Snyder, R., 2016. Experimental assessment of lionfish removals to mitigate reef
437   fish community shifts on northern Gulf of Mexico artificial reefs. Mar. Ecol. Prog. Ser. 558, 207–
438   221. https://doi.org/10.3354/meps11898
439   Dahl, K.A., Patterson, W.F., 2020. Movement, home range, and depredation of invasive lionfish revealed
440   by fine-scale acoustic telemetry in the northern Gulf of Mexico. Mar. Biol. 167, 111.
441   https://doi.org/10.1007/s00227-020-03728-4
442   Dahl, K.A., Portnoy, D.S., Hogan, J.D., Johnson, J.E., Gold, J.R., Patterson, W.F., 2018. Genotyping
443   confirms significant cannibalism in northern Gulf of Mexico invasive red lionfish, Pterois volitans.
444   Biol. Invasions 20, 3513–3526. https://doi.org/10.1007/s10530-018-1791-3
445   Dorenbosch, M., Grol, M.G.G., Christianen, M.J.A., Nagelkerken, I., Velde, G. van der, 2005. Indo-Pacific
446   seagrass beds and mangroves contribute to fish density and diversity on adjacent coral reefs.
447   Mar. Ecol. Prog. Ser. 302, 63–76. https://doi.org/10.3354/meps302063
448   ESR. 2009. OSCAR third deg. Ver. 1. PO.DAAC, CA, USA. Dataset accessed [2019-03-17]
449   at https://doi.org/10.5067/OSCAR-03D01

450   Ferreira, C., Luiz, O., Floeter, S., Lucena, M., Barbosa, M., Rocha, C., Rocha, L., 2015. First Record of
451   Invasive Lionfish (Pterois volitans) for the Brazilian Coast. PLoS ONE 10.
452   https://doi.org/10.1371/journal.pone.0123002

453   Fogg, A., Brown-Peterson, N., Peterson, M., 2017. Reproductive life history characteristics of invasive red
454   lionfish (Pterois volitans) in the northern Gulf of Mexico. Bull. Mar. Sci. 93.
455   https://doi.org/10.5343/bms.2016.1095
456   Fogg, A.Q., Evans, J.T., Peterson, M.S., Brown-Peterson, N.J., Hoffmayer, E.R., Ingram Jr., G.W., 2019.
457   Comparison of age and growth parameters of invasive red lionfish (Pterois volitans) across the
458   northern Gulf of Mexico. Fish. Bull. 117, 1–15. https://doi.org/10.7755/FB.117.3.1
459   Franco, A.D., Gillanders, B.M., Benedetto, G.D., Pennetta, A., Leo, G.A.D., Guidetti, P., 2012. Dispersal
460   Patterns of Coastal Fish: Implications for Designing Networks of Marine Protected Areas. PLOS
461   ONE 7, e31681. https://doi.org/10.1371/journal.pone.0031681
462   Green, S.J., Akins, J.L., Maljković, A., Côté, I.M., 2012. Invasive lionfish drive Atlantic coral reef fish
463   declines. PloS One 7, e32596.
464   Green, S.J., Dilley, E.R., Benkwitt, C.E., Davis, A.C.D., Ingeman, K.E., Kindinger, T.L., Tuttle, L.J., Hixon,
465   M.A., 2019. Trait-mediated foraging drives patterns of selective predation by native and invasive
466   coral-reef fishes. Ecosphere 10, e02752. https://doi.org/10.1002/ecs2.2752
467   Gress, E., Andradi-Brown, D.A., Woodall, L., Schofield, P.J., Stanley, K., Rogers, A.D., 2017. Lionfish
468   (Pterois spp.) invade the upper-bathyal zone in the western Atlantic. PeerJ 5, e3683.
469   https://doi.org/10.7717/peerj.3683
470   Harris, H.E., Fogg, A.Q., Gittings, S.R., Ahrens, R.N.M., Allen, M.S., Iii, W.F.P., 2020. Testing the efficacy of
471   lionfish traps in the northern Gulf of Mexico. PLOS ONE 15, e0230985.
472   https://doi.org/10.1371/journal.pone.0230985
473   Johnston, M., Purkis, S., 2015. A coordinated and sustained international strategy is required to turn the
474   tide on the Atlantic lionfish invasion. Mar. Ecol. Prog. Ser. 533, 219–235.
475   https://doi.org/10.3354/meps11399

476 Johnston, M.W., Bernard, A.M., Shivji, M.S., 2017. Forecasting lionfish sources and sinks in the Atlantic:
477   are Gulf of Mexico reef fisheries at risk? Coral Reefs 36, 169–181.
478   https://doi.org/10.1007/s00338-016-1511-3
479 Johnston, M.W., Purkis, S.J., 2011. Spatial analysis of the invasion of lionfish in the western Atlantic and
480   Caribbean. Mar. Pollut. Bull. 62, 1218–1226. https://doi.org/10.1016/j.marpolbul.2011.03.028
481 Jud, Z.R., Layman, C.A., 2012. Site fidelity and movement patterns of invasive lionfish, Pterois spp., in a
482 Florida estuary. J. Exp. Mar. Biol. Ecol. 414–415, 69–74. https://doi.org/10.1016/j.jembe.2012.01.015

483 Kalish, J.M., 1991. Oxygen and carbon stable isotopes in the otoliths of wild and laboratory-reared
484   Australian salmon (Arripis trutta). Mar. Biol. 110, 37–47. https://doi.org/10.1007/BF01313090
485 Kimball, M.E., Miller, J.M., Whitfield, P.E., Hare, J.A., 2004. Thermal tolerance and potential distribution
486   of invasive lionfish (Pterois volitans/miles complex) on the east coast of the United States. Mar.
487   Ecol. Prog. Ser. 283, 269–278.
488 Kitchens, L.L., Rooker, J.R., Reynal, L., Falterman, B.J., Saillant, E., Murua, H., 2018. Discriminating among
489   yellowfin tuna Thunnus albacares nursery areas in the Atlantic Ocean using otolith chemistry.
490   Mar. Ecol. Prog. Ser. 603, 201–213. https://doi.org/10.3354/meps12676
491 Laegdsgaard, P., Johnson, C., 2001. Why do juvenile fish utilise mangrove habitats? J. Exp. Mar. Biol.
492   Ecol. 257, 229–253. https://doi.org/10.1016/S0022-0981(00)00331-2
493 Lepak, J.M., Kraft, C.E., Weidel, B.C., 2006. Rapid food web recovery in response to removal of an
494   introduced apex predator. Can. J. Fish. Aquat. Sci. 63, 569–575. https://doi.org/10.1139/f05-248
495 Lesser, M.P., Slattery, M., 2011. Phase shift to algal dominated communities at mesophotic depths
496   associated with lionfish (Pterois volitans) invasion on a Bahamian coral reef. Biol. Invasions 13,
497   1855–1868. https://doi.org/10.1007/s10530-011-0005-z
498 Michener, R., Lajtha, K., 2008. Stable Isotopes in Ecology and Environmental Science. John Wiley & Sons.
499 Morris, J.A., Akins, J.L., 2009. Feeding ecology of invasive lionfish (Pterois volitans) in the Bahamian
500   archipelago. Environ. Biol. Fishes 86, 389–398. https://doi.org/10.1007/s10641-009-9538-8
501 Morris Jr, J.A., Whitfield, P.E., 2009. Biology, ecology, control and management of the invasive Indo-
502   Pacific lionfish: an updated integrated assessment.
503 Mumby, P.J., Edwards, A.J., Ernesto Arias-González, J., Lindeman, K.C., Blackwell, P.G., Gall, A.,
504   Gorczynska, M.I., Harborne, A.R., Pescod, C.L., Renken, H., C. C. Wabnitz, C., Llewellyn, G., 2004.
505   Mangroves enhance the biomass of coral reef fish communities in the Caribbean. Nature 427,
506   533–536. https://doi.org/10.1038/nature02286
507 Nagelkerken, I., van der Velde, G., Gorissen, M.W., Meijer, G.J., Van't Hof, T., den Hartog, C., 2000.
508   Importance of Mangroves, Seagrass Beds and the Shallow Coral Reef as a Nursery for Important
509   Coral Reef Fishes, Using a Visual Census Technique. Estuar. Coast. Shelf Sci. 51, 31–44.
510   https://doi.org/10.1006/ecss.2000.0617
511 Nuttall, M., 2014. Lionfish (Pterois volitans [Linnaeus, 1758] and P. miles [Bennett, 1828]) records within
512   mesophotic depth ranges on natural banks in the Northwestern Gulf of Mexico. BioInvasions
513   Rec. 3, 111–115. https://doi.org/10.3391/bir.2014.3.2.09
514 Paillon, C., Wantiez, L., Kulbicki, M., Labonne, M., Vigliola, L., 2014. Extent of Mangrove Nursery Habitats
515   Determines the Geographic Distribution of a Coral Reef Fish in a South-Pacific Archipelago. PLOS
516   ONE 9, e105158. https://doi.org/10.1371/journal.pone.0105158
517 Pella, J., Masuda, M., 2001. Bayesian methods for analysis of stock mixtures from genetic characters.
518   Fish. Bull. 99, 151–151.
519 Pracheil, B.M., Lyons, J., Hamann, E.J., Short, P.H., McIntyre, P.B., 2019. Lifelong population connectivity
520   between large rivers and their tributaries: A case study of shovelnose sturgeon from the
521   Mississippi and Wisconsin rivers. Ecol. Freshw. Fish 28, 20–32.
522   https://doi.org/10.1111/eff.12423

523 Reis-Santos, P., Tanner, S.E., França, S., Vasconcelos, R.P., Gillanders, B.M., Cabral, H.N., 2015.
524   Connectivity within estuaries: An otolith chemistry and muscle stable isotope approach. Ocean
525   Coast. Manag., Coastal systems under change 118, 51–59.
526   https://doi.org/10.1016/j.ocecoaman.2015.04.012
527 Schofield, P., 2010. Update on geographic spread of invasive lionfishes (Pterois volitans [Linnaeus, 1758]
528   and P. miles [Bennett, 1828]) in the Western North Atlantic Ocean, Caribbean Sea and Gulf of
529   Mexico. Aquat. Invasions 5, S117–S122. https://doi.org/10.3391/ai.2010.5.S1.024
530 Semmens, B., Buhle, E., Salomon, A., Pattengill-Semmens, C., 2004. A hotspot of non-native marine
531   fishes: evidence for the aquarium trade as an invasion pathway. Mar. Ecol. Prog. Ser. 266, 239–
532   244. https://doi.org/10.3354/meps266239
533 Smith, S.J., Campana, S.E., 2010. Integrated stock mixture analysis for continous and categorical data,
534   with application to genetic–otolith combinations. Can. J. Fish. Aquat. Sci. 67, 1533–1548.
535   https://doi.org/10.1139/F10-078
536 Spiegelhalter, D., Thomas, A., Best, N., Lunn, D., Unit, M.B., 2001. WinBUGS user manual, in: United
537   Kingdom: MRC Biostatistics Unit, Institute of Public Health.
538 Stevens, P.W., Fox, S.L., Montague, C.L., 2006. The interplay between mangroves and saltmarshes at the
539   transition between temperate and subtropical climate in Florida. Wetl. Ecol. Manag. 14, 435–
540   444. https://doi.org/10.1007/s11273-006-0006-3
541 Switzer, T.S., Tremain, D.M., Keenan, S.F., Stafford, C.J., Parks, S.L., Jr, R.H.M., 2015. Temporal and
542   Spatial Dynamics of the Lionfish Invasion in the Eastern Gulf of Mexico: Perspectives from a
543   Broadscale Trawl Survey. Mar. Coast. Fish. 7, 1–8.
544   https://doi.org/10.1080/19425120.2014.987888
545 Tamburello, N., Côté, I.M., 2015. Movement ecology of Indo-Pacific lionfish on Caribbean coral reefs and
546   its implications for invasion dynamics. Biol. Invasions 17, 1639–1653.
547   https://doi.org/10.1007/s10530-014-0822-y
548 Thibault, I., Hedger, R.D., Dodson, J.J., Shiao, J.-C., Iizuka, Y., Tzeng, W.-N., 2010. Anadromy and the
549   dispersal of an invasive fish species (Oncorhynchus mykiss) in Eastern Quebec, as revealed by
550   otolith microchemistry. Ecol. Freshw. Fish 19, 348–360. https://doi.org/10.1111/j.1600-
551   0633.2010.00417.x
552 Travis, J.M.J., Murrell, D.J., Dytham, C., 1999. The evolution of density–dependent dispersal. Proc. R. Soc.
553   Lond. B Biol. Sci. 266, 1837–1842. https://doi.org/10.1098/rspb.1999.0854
554 Tscharntke, T., Rand, T.A., Bianchi, F.J.J.A., 2005. The landscape context of trophic interactions: insect
555   spillover across the crop—noncrop interface. Ann. Zool. Fenn. 42, 421–432.
556 Weidel, B.C., Josephson, D.C., Kraft, C.E., 2007. Littoral Fish Community Response to Smallmouth Bass
557   Removal from an Adirondack Lake. Trans. Am. Fish. Soc. 136, 778–789.
558   https://doi.org/10.1577/T06-091.1
559 Weidman, C.R., Millner, R., 2000. High-resolution stable isotope records from North Atlantic cod. Fish.
560   Res. 46, 327–342. https://doi.org/10.1016/S0165-7836(00)00157-0
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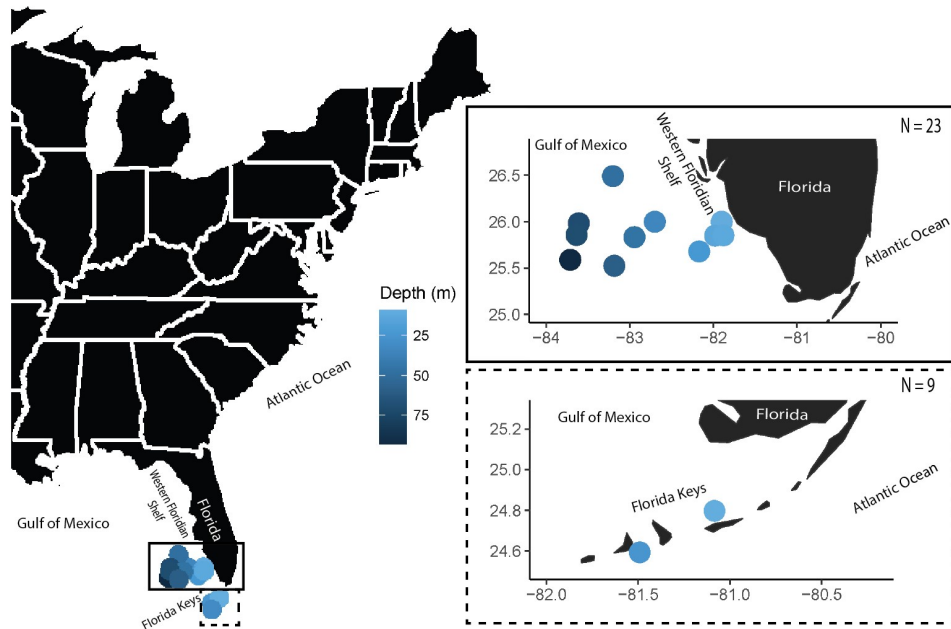
569   **Tables and Figures:**

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Figure 1. Map of sites and depths sampled throughout Floridian coastal waters. There are 11 sites throughout the Western Floridian Shelf (WFS) and 2 within the Florida Keys.
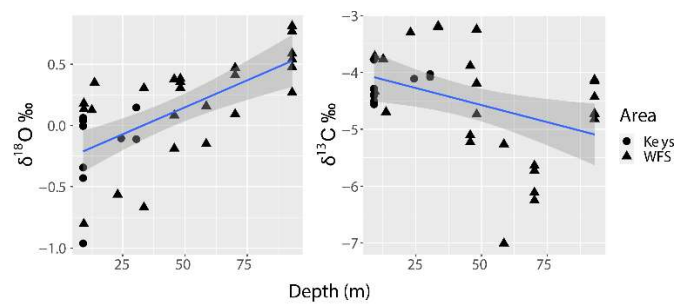
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Figure 2. Isotopic profiles for both δ18O and δ13C. δ18O increases with increas ing depth (R2 = 0.42, F(1,33) = 23.95, p < 0.001). δ13C decreases with increas ing depth (R2 = 0.17, F(1,33) = 8.857, p = 0.013). These isotopic compositions were calculated from rim isotopes and represent the range of isotopes that are found in each depth step.
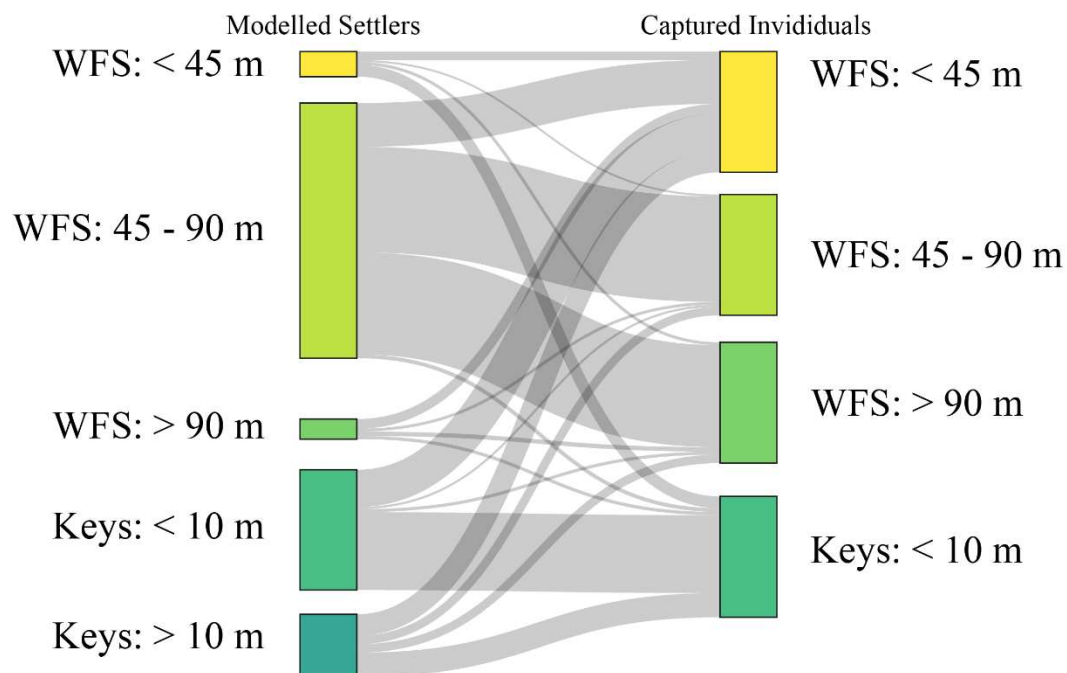
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Figure 3. The connections between modelled settlers and captured individuals represent the modelled proportion of settlers from each depth that disperse across the studied regions. The captured individuals represent the adult fish from which otoliths were sampled.
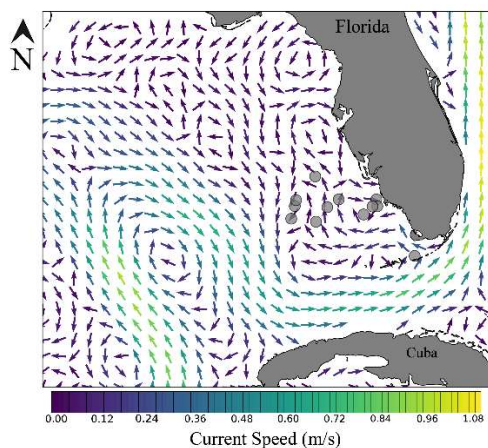
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Figure 4. The currents throughout the Gulf of Mexico, highlighting the Florida Loop Current and the many slower-movings currents around it. Gray points indicate sampling locations. Data Source: ESR 2009.

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Table 1. Standard deviation of replicate samples.

	δ^{13}C (‰)	δ^{18}O (‰)
Otolith Core	0.45	0.28
Otollith Rim	0.61	0.03
NBS-19 Standard	0.03	0.06

Table 2. Modeled contribution of each sampled region to populations across depths, including 90% confidence intervals (CI). Analysis was done through Bayesian Stock Mixture Analysis, an MCMC method (iterations = 50000).

		Predicted settlement								
		WFS: 0 - 45 m			WFS: 45 - 90 m			WFS: > 90 m		
			90% CI			90% CI			90% CI	
		Mean	5%	95%	Mean	5%	95%	Mean	5%	95%
Location of Capture	WFS: 0 - 45 m	7.04	0.00000	36.55	36.249	0.00664	82.4505	8.08	0.00003	40.04
	WFS: 45 - 90 m	1.60	0.00001	7.76	87.326	7.51565	99.8005	2.30	0.00001	10.71
	WFS: > 90 m	2.41	0.00000	12.25	84.477	11.9815	99.6005	3.58	0.00000	16.35
	Keys: 0 -10 m	9.7	0.00001	58.86	3.152	0.00001	15.4645	2.68	0.00001	14.26

		Keys: 0 -10 m			Keys: > 10 m		
			90% CI			90% CI	
		Mean	5%	95%	Mean	5%	95%
Location of Capture	WFS: 0 - 45 m	30.83	0.00188	82.93	17.79	0.00003	74.8
	WFS: 45 - 90 m	1.80	0.00000	8.97	6.98	0.00001	81.48
	WFS: > 90 m	2.53	0.00001	13.18	7.00	0.000000	57.78
	Keys: 0 -10 m	64.42	0.01395	99.16	20.05	0.00001	95.19