1   **Subsidy Accessibility Drives Asymmetric Food Web Responses**

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12   **Abstract (max 350 words)**

13   Global change is fundamentally altering flows of natural and anthropogenic subsidies across space

14   and time. After a pointed call for research on subsidies in the 1990s, an industry of empirical work

15   has documented the ubiquitous role subsidies play in ecosystem structure, stability and function.

16   Here, we argue that physical constraints (e.g., water temperature) and species traits can govern a

17   species' accessibility to resource subsidies, and that these physical constraints have been largely

18   overlooked in the subsidy literature. We examined the input of a high quality, point-source

19   anthropogenic subsidy into a recipient freshwater lake food web (i.e., released net-pen aquaculture

20   feed in Parry Sound, Lake Huron), to demonstrate the importance of subsidy accessibility in

21   governing recipient whole food web responses. By using a combined bio-tracer approach, we

22   detect a gradient in accessibility of the anthropogenic subsidy within the surrounding food web

23   driven by the thermal tolerances of three constituent species. This thermally-driven accessibility

24   gradient drives asymmetrical changes in food web structure, effectively rewiring the recipient lake

25   food web and altering patterns in secondary production with yet unknown stability consequences.

26   Since aquaculture is predicted to increase significantly in coming decades to support growing

27   human populations, and global change is altering temperature regimes, then this form of food web

28   alteration may be expected to occur frequently. We argue that subsidy accessibility is a key

29   characteristic of recipient food web interactions that must be considered when trying to understand

30   the impacts of subsidies on ecosystem stability and function under continued global change.

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32   **Keywords**: Subsidy accessibility, anthropogenic subsidies, food web structure, bio-tracers, stable

33   isotopes, fatty acids, net-pen aquaculture, lake

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Introduction

37 Ecosystems are intrinsically connected through space and time by flows of energy (i.e.,

38 subsidies) that ultimately help govern the receiving ecosystem's stability and function.

39 Foundational work by empirical ecologists in the late 1990's demonstrated the ubiquity of

40 natural subsidies (e.g., nutrients, detritus, prey) connecting landscapes across a broad range of

41 spatial and temporal scales (Polis & Strong, 1996; Polis & Winemillar 1996; Polis et al., 1997;

42 Sears et al., 2004). This empirical work led to theory demonstrating that subsidies are pivotal in

43 determining the stability and community composition of recipient food webs (Polis et al., 1997;

44 Huxel & McCann, 1998; Sears et al., 2004, Takimoto et al. 2002). While subsidies occur

45 naturally, a growing human population is increasing the prevalence of anthropogenic subsidies

46 to natural ecosystems (e.g., agricultural nutrient run-off, sewage, net-pen aquaculture waste).

47 Akin to natural subsidies, anthropogenic subsidies also have the potential to influence recipient

48 food web dynamics (DeBruyn et al., 2004; Rodewald et al., 2011; Newsom et al., 2015; Singer

49 et al., 2016; Lee et al., 2018; Johnson et al., 2018; DeBruyn et al., 2020). As food web dynamics

50 govern whole ecosystem stability and function (de Ruiter et al., 1995; Neutel et al., 2002), it is

51 imperative to understand how recipient food webs respond to both natural and anthropogenic

52 subsidies under continued global change.

53 It is now well recognized that fluxes of subsidies play a major role across most

54 ecosystems (Leroux & Loreau, 2008) and also ought to significantly impact the stability and

55 functioning of recipient ecosystems (Huxel & McCann, 1998; Takimoto et al., 2002; Leroux &

56 Loreau, 2008). An industry of empirical and theoretical research has emerged since Polis and

57 others introduced subsidies to food web theory in the 1990s with researchers exploring the

58   various ways subsidies can impact recipient ecosystems. Research has concentrated on: i)

59   subsidy quality and quantity; ii) the temporal nature of subsidies (e.g., Nakano et al., 1999;

60   Nakano & Murakami, 2001; Sears et al., 2004); iii) the role of subsidies in driving trophic

61   cascades (Huxel & McCann, 1998), and; iv) the roles space and ecosystem type play in

62   governing subsidy flow rate, assimilation, and magnitude of resultant impacts on food web

63   dynamics (Leroux & Loreau, 2008). While this research has made major progress, surprisingly

64   little work has looked at the role of species traits (e.g., thermal preference) and physical habitat

65   characteristics (e.g., water temperature) in determining subsidy accessibility, and how this may

66   govern whole food web responses. Since accessibility may allow us to better understand how

67   subsidies integrate into whole recipient webs this is a major gap in subsidy research.

68   While subsidies are generally thought to be ubiquitous in nature, it is less understood

69   how the physical location of a subsidy within a habitat or ecosystem impacts its ability to be

70   accessed and thus assimilated by members of the local food web. Subsidy accessibility, defined

71   as the degree of availability of subsidies to recipient species based on their physiological (e.g.,

72   thermal tolerance) and physical habitat limitations, can drive differential increases in

73   productivity throughout the receiving food web. According to optimal foraging theory, if

74   capable, species will alter their foraging behaviour to access the highest density/quality resource

75   available (i.e., also referred to as the Birdfeeder Effect at the community level (Eveleigh et al.,

76   2007)). However, species may be limited in their ability to respond to these changes in local

77   resource densities by traits, such as mobility and thermal tolerance. For example, subsidies

78   entering the nearshore zone of a temperate lake may be expected to be most easily accessed by

79   warm adapted fishes and least accessible to cold adapted species. Therefore, in the example

80   above, we may expect to see the greatest alteration in diet towards subsidies in warm water

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81 species and reduced or absent alteration in diet in cold water species. This type of asymmetrical

82 subsidy accessibility and uptake can in turn drive differential changes in productivity and thus

83 food web structure throughout whole recipient ecosystem. Here, we combine the ideas that

84 species optimally forage within their physical and physiological capabilities and the asymmetric

85 impacts of spatial subsidies on receiving food webs, to suggest that species' ability to access

86 subsidies can drive differential (or asymmetric) changes in food web productivity and structure.

87 In this study, we broadly examine the dispersal of a point-source anthropogenic subsidy into a

88 temperate lake food web that contains dominant mobile, generalist top predators from each of the three

89 thermal guilds (i.e., a gradient in trait responses from cold- to coo-l to warm- water fish) (Magnuson et

90 al., 1990). We build off empirical evidence of assimilation of a high-quality anthropogenic subsidy in

91 our study system, released net-pen aquaculture feed (Johnson et al., 2018), to demonstrate how

92 accessibility to this subsidy is key in governing the receiving food web's response to this novel

93 anthropogenic energy source. Differential accessibility throughout the recipient food web has the

94 potential to differentially alter productivity and thus asymmetrically alter food web structure (e.g.,

95 differential changes in food chain length). As changes in food web structure can drive alterations in

96 food web dynamics, this is a key first step in understanding how subsidy accessibility may ultimately

97 influence food web stability. Here, we use a combined bio-tracer approach to trace the fate of the

98 anthropogenic subsidy throughout the lake food web, and then combine hydroacoustic, fish biomass,

99 and stable isotope data to detect asymmetrical shifts in food web productivity and structure (i.e., food

100 chain length) across a thermal gradient.

101 **Methods**

102 *Site Description*

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103   Parry Sound, located in Georgian Bay of Lake Huron, was chosen as our study site to

104   investigate how accessibility to a point-source anthropogenic subsidy influenced assimilation

105   into a surrounding food web and alterations in subsequent food web productivity and structure.

106   This location presents an ideal study system to address this question as there is a large

107   consistent, long-term, high quality point-source anthropogenic subsidy (i.e., released net-pen

108   feed & waste from Aqua-Cage Fisheries Ltd.). Rainbow trout are reared within the Aqua-Cage

109   net-pens and are fed daily from feed boats that move between cages from ~9:00-16:00 each day

110   (Kana Upton, Aqua-Cage Fisheries Ltd., Pers. Comm). Feed consists of pelleted food

111   manufactured from both aquatic and terrestrial energy sources (see Appendix1: Table S1 and

112   Table S2 for composition of the fish feeds). Parry Sound undergoes thermal stratification during

113   summer, which creates a natural thermal gradient from the near-shore, warm littoral zones (that

114   are more accessible to fishes with warmer thermal preferences) to the off-shore, cold pelagic

115   zones (that are more accessible to fishes with colder thermal preferences). The location of the

116   point-source subsidy in the deep, cold region of the lake thus sets up a natural gradient of

117   accessibility to the net-pen feed from most accessible (cold-water species), intermediate

118   accessibility (cool-water species), to least accessible (warm-water species).

119   To determine if any changes in diet and food web structure in Parry Sound were in fact

120   driven by differential assimilation of net-pen feed, we selected multiple control sites around

121   Georgian Bay, Lake Huron (see Appendix 1: Figure S1 for map). Control sites were selected by

122   the following criteria: no presence of off-shore point-source anthropogenic subsidy, far distance

123   to ensure no mixing of populations between subsidy and control sites, and presence of

124   comparable species. One control site was sampled in 2016 (Shawaniga/Sturgeon Bay) and three

125   control sites were sampled in in 2017 (Shawniga/Sturgeon Bay, Key Harbour, and Dyers Bay).

126   *Fish & Baseline Sampling*

127   Muscle tissue samples were collected from fish and whole invertebrates for stable

128   isotope (SI) and fatty acid (FA) analysis from both Parry Sound and control sites in 2016 and

129   2017. Fish and invertebrate species were selected to recreate a tri-trophic generalist lake food

130   web that contains a dominant generalist top predator in all three thermal guilds (lake trout (cold),

131   walleye (cool), smallmouth bass (warm)), cold off-shore (alewife & rainbow smelt) and cool

132   near-shore (yellow perch) forage fish, and off-shore/near-shore baseline invertebrates. Figure 1

133   shows conceptual diagram of the tri-trophic generalist lake food web sampled.

134   Fish samples were collected using Ontario multi-mesh gill nets following a modified

135   broad-scale monitoring (BsM) protocol of the fish community (Sandstrom et al., 2013) and

136   supplemented with targeted angling. Both overnight and daytime sets were employed in the

137   sampling protocol. All gill nets were set for a duration of ~12 h before retrieving fish. Weight

138   (g), length (mm), and two muscle tissue plugs from behind the dorsal fin were collected from

139   fish. Non-predatory insect larvae (i.e., *Ephemeroptera* larvae), snails and mussels (*Unionidae*

140   *sp.,* and *Dreissena polymorpha*) were collected from each sampling site to provide baseline

141   stable isotope values. Additionally, feed and muscle tissue from farmed rainbow trout was also

142   collected each year Aqua-Cage Fisheries in Parry Sound. All samples were stored at -20˚C after

143   collection until further analysis.

144   One muscle tissue plug from all fish, feed, and baseline samples collected were prepared

145   for stable isotope analysis. Samples were thawed in the lab and dried at 60 °C in a drying oven

146   for 48 h. Once dried, they were individually ground using a mortar and pestle and scooped into a

147   labelled centrifuge tube. The samples were sent to the University of Windsor GLIER (Windsor,

148   ON, Canada) laboratories for carbon and nitrogen isotopic analysis. Muscle tissues from a subset

149   of the fish collected were sent for fatty acid analysis, along with feed and baseline samples.

150   Frozen samples were delivered to Ryerson University – The Arts Lab (Toronto, ON, Canada) in

151   2016, and both the Laboratory of Aquatic Sciences (Chicoutimi, QC, Canada) and Lipid

152   Analytical Services (Guelph, ON, Canada) in 2017. All labs conducted fatty acid analysis using

153   a combination of Bligh & Dyer and Morrison & Smith methods (Bligh & Dyer, 1959; Morrison

154   & Smith, 1964). Individual FA weights (ug/g) were converted to a % FA composition and fatty

155   acids with >1% presence were retained for analysis.

156   *Detecting Assimilation of Net-Pen Subsidy into Surrounding Food Web*

157   To determine which fatty acids were key indicators of net-pen aquaculture feed (i.e.,

158   fatty acids that are known to be primarily dietary-derived and are significantly higher or lower in

159   the feed than in natural surrounding prey sources) a principal components analysis (PCA) was

160   conducted on fatty acids with >1% average proportions present in forage fish, feed, and baseline

161   fatty acid profiles from all sites. Fatty acid % were standardized to a mean of zero and unit

162   variance prior to their inclusion in the PCA, and separate PCAs were performed for 2016 and

163   2017 because different fish feeds with different compositions were used in each year (see

164   Supplementary Table 1, Table 2, & Figure 2). Fatty acids with significant loadings (> +/-0.3)

165   along PC1 (indicating significantly higher amounts in feed than natural food sources) were

166   retained as Feed Indicator Fatty Acids (FIFA).

167   FIFA composition of each individual species was then compared between net-pen and

168   control sites using PCAs. The goal of this analysis was to determine if there was distinct

169   clustering of net-pen and control populations driven by the FIFA. To test whether the distance

170   between the net pen and control sites increased from warm to cool to cold water species

171   (reflecting increasing access to and assimilation of the net-pen subsidies), we compared the

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172   Euclidean distance between the centroids of the clusters within the PCA. PERMANOVA was

173   used to test for significant differences in the % FIFA composition between net-pen and control

174   sites for each species (Anderson, 2017). As this technique yields pseudo-F statistics, P-values

175   can be calculated using permutations that yield P-values with significance considered for

176   P<0.05. Due to the small and differing sample sizes, we conducted 100,000 permutations to

177   compute a nonparametric PERMANOVA (Steeves et al., 2018). Univariate boxplot comparisons

178   were used to identify which FIFA were driving significant differences in FIFA profiles.

179   *Detecting Local Changes in Recipient Food Web Biomass Densities*

180   Hydroacoustic surveys were conducted in 2017 to determine the horizontal and vertical

181   spatial distribution of fish surrounding the net-pen facility to detect local aggregations of fish

182   densities surrounding the point-source subsidy input, indicating potential local increases in

183   productivity. One day and one night survey was conducted on July 5 (day survey from 14:30-

184   19:00) and July 6 (night survey from 23:45 - 04:00) 2017 following a 2000 m transects leading

185   away from the net-pen facility (see map inset in Fig. 6). All surveys were conducted following

186   the standard operating procedures outlined in Parker-Stetter et al. (2009) (see Appendix 2 for

187   method details).

188   To determine if there were significantly higher densities of fish closer to the point-source

189   subsidy input location, Echoview acoustic post-processing software version 7.1.36.30718 was

190   used to convert the cleaned fish count data into fish density values (see Supplementary Methods

191   for details). Fish density values were extracted from Echoview and linear regressions (a = 0.05)

192   were conducted to compare fish density to distance from net-pen for both night and day surveys.

193   Non-linear data was log transformed prior to statistical analysis.

194   *Detecting Regional Changes in Receiving Food Web Biomass*

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195   Fish biomass data from five sites across Georgian Bay (Fig. 7) were obtained from the

196   MNRF's 2017 Broad-scale Monitoring program (see UGLMU, 2018 for detailed collection

197   methods), to determine if the off-shore net-pen subsidy in Parry Sound drove asymmetrical

198   increases in the cold water, off-shore (pelagic) biomass. Biomass proportions of all species

199   caught in the 2017 BsM sampling was extracted from the MNRF Annual Report of Fisheries

200   Assessment Projects Conducted on Lake Huron, 2017 and sorted into a thermal guild based on

201   their thermal preference (Hasnian et al., 2018). Species with final temperature preferendum

202   (FTP, the temperature that fish gravitate towards when provided with a broad range of

203   temperatures (Hasnain et al., 2018)) <17˚C were classified as cold, 17-25˚C were classified as

204   cool, and >25˚C were classified as warm (Coker et al., 2001). Total proportion of each thermal

205   guild and proportion of each individual species was calculated. Distribution of total biomass

206   across thermal guilds and species was then compared to see if Parry Sound had

207   disproportionately higher biomass in the cold, off-shore thermal guild relative to sites with no

208   net-pen facilities.

209   *Detecting Changes in Receiving Food Web Structure*

210   To detect alterations in food web structure driven by the point-source subsidy in Parry Sound, we

211   compared the trophic position of the representative food web collected from Parry Sound and control

212   sites in 2016 and 2017 using a carbon and nitrogen two-source stable isotope mixing model (Post,

213   2002). There is little to no fractionation of $\delta^{13}C$ and is used to track basal energy sources (i.e., %

214   littoral carbon use), while $\delta^{15}N$ fractionates from resource to consumer and thus is used to track

215   trophic position. To increase the number of control sites, additional $\delta^{15}N$ and $\delta^{13}C$ data was obtained

216   for comparable species from numerous control sites around Lake Huron in 2017 as part of various

217   OMNRF research projects (UGLMU, 2018) (see Appendix 1: Figure S1 for map of sites). All

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218   information regarding collection of these samples can be found in the OMNRF Annual Report of

219   Fisheries Assessment Projects Conducted on Lake Huron, 2017 (UGLMU, 2018). Non-lipid corrected

220   C and N values were used as the majority of C:N were low (81% < 3.5 and 92% < 4) (Post et al.,

221   2007).

222   The following equations were used to calculate trophic position for the mobile, generalist

223   top predators, which incorporate coupling of pelagic and littoral energy channels ((Post, 2002):

224     $$TP_{Predator} = 2 + (\delta^{15}N_{predator} - (\delta^{15}N_{pelagic\ baseline} * (1-\alpha) + \delta^{15}N_{littoral\ baseline} * \alpha))/ 3.4$$

225     $$\alpha = (\delta^{13}C_{predator} - \delta^{13}C_{pelagic\ baseline})/ (\delta^{13}C_{littoral\ baseline} - \delta^{13}C_{pelagic\ baseline})$$

226   where $\delta^{15}N_{predator}$ and $\delta^{13}C_{predator}$, are the nitrogen and carbon stable isotope values for predatory

227   fish collected (lake trout, walleye, smallmouth bass) and $\delta^{15}N_{pelagic\ baseline}$, $\delta^{13}C_{pelagic\ baseline}$, , $\delta^{15}N$

228     $_{littoral\ baseline}$, $\delta^{13}C_{littoral\ baseline}$ are the nitrogen and carbon stable isotope values for

229   mussels/zooplankton and mayflies respectively. Coupling is incorporated into trophic position

230   calculation through α, which weighs the trophic position estimate according to baseline

231   contributions (Vander Zanden et al., 1999; Vander Zanden et al., 2000).

232   Trophic position of forage fish was calculated using the following equation, which does

233   not incorporate coupling (Post, 2002):

234     $$TP_{Intermediate\ Consumer} = 2 + (\delta^{15}N_{prey\ fish} - (\delta^{15}N_{baseline})) /3.4$$

235   where $\delta^{15}N_{prey\ fish}$ and $\delta^{15}N_{baseline}$ are the nitrogen stable isotope values of forage fish and

236   associated baseline (e.g., littoral forage fish would use littoral baselines). To test for significant

237   differences in trophic position between subsidy and control sites, ANOVA with TukeyHSD

238   post-hoc analysis was conducted (a = 0.05).

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239   All statistical analysis was completed using the R statistical computing package, version

240   3.6.1 (R Core Team 2019). Full raw data and associated R code will be available on GitHub

241   upon manuscript acceptance.

242

243   **Results**

244   *Net-pen Aquaculture Feed Indicator Fatty Acid Selection*

245   PCA of all fatty acids (FA) composing >1% of total FA composition for each forage fish,

246   baseline, and feed sample from net-pen and control sites in 2016 and 2017 successfully

247   separated net-pen feed sources from natural biota across PC1 (Fig 2. (a), (b)). PC1 explained

248   38.3% of total variation of fatty acid composition in 2016 and 38.8% in 2017. This variance was

249   primarily driven by 3 FA in 2016 (higher % in feed: 18:2n6, 18:1n9, 20:1n9), and 4 FA in 2017

250   (higher % in feed: 18:2n6, 18:3n3, 18:1n9, 16:1n7), which all loaded significantly onto PC1 and

251   in the direction of the net-pen feed (i.e., PC score > 0.3). These FA were retained as 'feed

252   indicator fatty acids' (FIFA) to be used in subsequent analysis comparing individual species

253   FIFA compositions between net-pen and control sites.

254   *Asymmetrical Uptake of Net-Pen Subsidies Across Thermal Guilds*

255   PCA on FIFA profiles of individual species between net-pen and control sites in 2016 and

256   2017 show differences in the degree of separation of net-pen and control populations among thermal

257   guild and trophic level. The cold-water top predator (Fig. 4(a; *i*)) and forage fish (Fig. 4(b; *i*)), as well

258   as the cool-water forage fish (Fig. 3(d; *i*); Fig. 4(c; *i*)) demonstrate the highest degree of separation

259   between net-pen and control populations. The cool-water top predator demonstrates some degree of

260   separation between net-pen and control populations (Fig. 3(a; *i*); Fig. 4(c; *i*)), and the warm-water top

261   predator shows some separation of populations in 2016, but a complete overlap with control

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262   populations in 2017 (Fig. 3(b; *i*); Fig. 4(d; *i*)). Euclidean distances between cluster centroids of net-pen

263   and control populations demonstrate a negative relationship between species thermal preference (i.e.,

264   FTP) and centroid distance, where cold water species tend to have the greatest distance between

265   populations and warm water species the least (Figure 5).

266   PERMANOVA comparing the % FIFA composition between net-pen and control populations

267   of each species shows that cold-water top predators ($p = 0.0036$), cold-water forage fish ($p = 0.0304$)

268   and cool water forage fish ($p = 0.0343$) have the most significantly different FIFA profiles relative to

269   control sites in 2017. The 2016 cool-water forage fish population had the most significant difference in

270   FIFA populations across both years ($p = 0.0008$). In the cold-water top predator and forage fish these

271   significant differences are driven by higher levels of linoleic acid (18:2n6) and linolenic acid (18:3n3)

272   in net-pen populations relative to control (Fig. 4, (a; *ii*); (b; *ii*)). In the cool-water forage fish,

273   significant differences are driven by higher levels of all FIFA in net-pen populations for both 2016 and

274   2017. The cool-water top predator (i.e., Walleye) net-pen associated FIFA composition was

275   significantly different from control populations in 2017 ($p = 0.0384$) but not in 2016 ($p = 0.378$).

276   Consistent with cold-water species, Walleye also demonstrated higher levels of linoleic acid (18:2n6)

277   and linolenic acid (18:3n3) in net-pen populations relative to control in 2017 (Fig. 4, (c; *ii*)). Despite

278   not demonstrating significant differences in overall FIFA profiles in 2016, univariate boxplots show

279   Walleye still exhibited higher levels of FIFA relative to the control population (Fig. 3, (a; *ii*)), The

280   warm-water top predator (i.e., Smallmouth bass) showed no difference in FIFA profiles in 2017 ($p = $

281   0.633) and a marginally significant difference in all 3 FIFA in 2016 ($p = 0.056$). Univariate boxplots

282   results show that this marginal significance was driven by higher levels of all 3 FIFA in 2016 net-pen

283   associated smallmouth bass.

284   *Asymmetric Changes in Recipient Food Web Productivity*

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285   Linear regression of fish density surrounding net-pen facility demonstrate increased local

286   aggregations of fish density closer to the net-pen facility than farther away (Figure 6). The strength of

287   aggregation around the net-pen was stronger at night than during the day. While both day and night

288   surveys show decreases in fish density with increasing distance from the net-pen, only the night

289   transect was significant (Night: p = 0.0089; Day: p = 0.4595). The net-pen is located in 75 m of water

290   and the transect led away from shore, thus we can eliminate the possibility that this effect is caused by

291   a near-shore, littoral increase in fish densities.

292   Comparison of biomass distribution of cold, cool and warm water species in net-pen (Parry

293   Sound and South Bay) vs. 4 control sites across Georgian Bay, demonstrate Parry Sound and South

294   Bay have higher proportions of cold-water biomass than control sites (Figure 7), which was not

295   reflected in the cool- and warm-water guilds. A large proportion of the cold water biomass in Parry

296   Sound is comprised of Lake Trout (12.46%), Cisco (2.94%) and Alewife (1.21%), which are three

297   cold-water species that were much less abundant throughout the Georgian Bay (see Supplementary

298   Table 3 for % biomass of species at sites across Georgian Bay).

299   *Asymmetric Changes in Recipient Food Web Structure*

300   The ANOVAs comparing trophic position of cold, cool, and warm water species collected

301   across Lake Huron found that the cold-water top predator and cold water forage fish have significantly

302   higher trophic positions in Parry Sound relative to any other control site in Lake Huron (F = 40.328, p

303   = <0.001; F = 48.582, p = <0.001, respectively) (Figure 6 (a), (d)). Cool-water top predators and

304   forage fish exhibited significant differences in trophic position between sites across Lake Huron (F =

305   15.352. p = <0.001; F = 18.878, p = <0.001, respectively), however this significance was not driven by

306   higher trophic position in Parry Sound relative to all control sites (Figure 6 (b),(e)). Warm-water top

307   predators did not exhibit significant differences between net-pen and control sites (F=2.948, p =

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308   0.06219) (Fig. 6(c).) It should be noted that the MNRF data only included pelagic baselines, therefore

309   coupling was not possible to calculate and incorporate into trophic position calculation of MNRF

310   sampled generalist predators. Statistical outcomes were consistent regardless of if coupling was

311   incorporated or not, thus coupling was incorporated where possible to provide more accurate trophic

312   position estimates when able.

313   **Discussion**

314   Despite the fact that accessibility may fundamentally alter the influence of a subsidy on

315   resultant food web dynamics, surprisingly this aspect of subsidies remains relatively unexplored. Here,

316   we use a natural experiment to determine how species traits, particularly thermal tolerance, influenced

317   accessibility to an off-shore point-source anthropogenic subsidy, and in turn show that differential

318   accessibility drives asymmetric changes in food web productivity and structure. Our results suggest

319   that species traits can produce a gradient in subsidy accessibility, as cold-water adapted species show

320   the greatest evidence of subsidy consumption, and warm-water species demonstrate little to none.

321   Notably, our feeding results also scale up to show consistent asymmetric changes in productivity and

322   food web structure such that cold-water species, for example, consume most of the subsidy and have

323   elevated biomass and trophic position.

324   Recent work has argued that climate change can be expected to generally have asymmetrical

325   impacts on habitat (e.g., habitats differentially warmed by climate change) that lead to organismal

326   behaviour that asymmetrically rewires whole carbon pathways. Bartley et al (2018) argued that

327   differential habitat changes and species behaviour (traits) then drive wholesale food web rewiring. Our

328   results here suggest that such impacts of climate change rewiring may extend more broadly to other

329   aspects of global change. Specifically, here we showed that the placement of net-pen aquaculture in

330   cold-water habitats (i.e., a global change that is necessarily habitat dependent and so asymmetrical)

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331   produces stronger interactions as a function of species traits (i.e., thermal tolerance) and so

332   functionally rewires this ecosystem, altering food web structure and productivity with yet unknown

333   stability implications (Blanchard et al., 2015; Bartley et al. 2018). Like climate change, net-pen inputs

334   can differentially alter macrohabitats (littoral versus pelagic) and so drive asymmetric rewiring. It

335   remains to be seen whether human impacts that cross multiple habitats generally have asymmetrical

336   impacts, and if they do, this suggests a general rewiring result of global change on spatial food webs.

337   The use of fatty acids to trace specific prey items through food webs has provided a powerful

338   tool for understanding how subsidies move through and are utilized by the surrounding ecosystem.

339   Fatty acid composition analysis has been widely used in the aquaculture literature to identify the

340   presence of net-pen feed in the diet of surrounding fish species in both marine and freshwater systems

341   (Kullman et al., 2009; Fernandez-Jover et al., 2011; Johnson et al., 2018). The presence of marine and

342   terrestrial derived protein and oil in fish feed differentiate the fatty acid composition of the feed from

343   natural food sources (Fernandez-Jover et al., 2011). Fatty acids are highly conserved throughout the

344   food web, which provides the ability to trace net-pen feed through a surrounding food web because

345   differences in fatty acid composition between net-pen and control populations is proportional to the

346   amount of feed consumed (Fernandez-Jover et al., 2011).

347   We demonstrated how subsidy accessibility, measured through amount of consumption of net-

348   pen feed, depends on species' traits (e.g., thermal tolerance). Cold-water mobile generalists and forage

349   fish had significant differences in feed indicator fatty acid (FIFA) composition relative to control

350   populations (little overlap in FIFA clusters, large centroid distance (Fig. 4; Fig. 5)). This suggests their

351   diet is significantly different from control diets due to the presence of feed. Cold-water forage fish

352   were less significantly different than the mobile generalist, which may be a factor of the different

353   dominant pelagic forage fish found in each locations (Alewife in Parry Sound, Rainbow Smelt in

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354   Dyer's Bay). In our study, cool-water forage fish had the most significant difference in FIFA profile in

355   2016, and while still significant in 2017, not as significant as the cold-water generalist and forage fish.

356   Cool-water mobile generalists' FIFA profiles were also significantly different from control

357   populations, however the signal was not as strong (centroid distance not as far, more cluster overlap)

358   as in the cold-water species. The cool-water generalist and forage fish also displayed higher variation

359   in the Parry Sound populations relative to controls and cold-water species. This suggests there may be

360   more individual variation in the cool-water guild, where some individuals strongly incorporate FIFA

361   and some were no different than other control fish. Warm-water mobile generalists demonstrated high

362   overlap between net pen and control sites, suggesting little to no feed uptake and low access to net-pen

363   subsidies in 2017. Marginally significant differences between net pen and control sites in 2016

364   indicate some individuals may have accessed net-pen subsidies that year. Some evidence of access in

365   2016 may be due to warmer temperatures that summer (Parry Sound Historical Weather Data,

366   Environment Canada), which may have made a larger warmer zone and thus able to forage farther and

367   potentially access net-pen resources. It should be noted that littoral baselines (i.e., mayflies) had

368   similar values of linolenic acid (18:3n3) and higher values of palmitoleic acid (16:1n7) relative to the

369   feed due to the input of terrestrial oils in the feed (Appendix 1: Figure S1). This may drive similarities

370   in FIFA profiles of littoral cool and warm water species; therefore, it is important to focus on fatty

371   acids that are significantly different from the feed as stronger feed indicators in these species. Here,

372   our results provide evidence of a gradient in net-pen feed uptake, that is correlated with the gradient in

373   thermal preferences, suggesting thermal tolerances limit the ability of surrounding species to access

374   point-source subsidies. Further, our results suggest the intriguing possibility that the population of

375   cold-water species (e.g. lake trout) generally respond to consume from the cold-water feed site, while

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376   more thermally generalized species (cool-water) show greater variation in response suggesting

377   individual variation in amount of feed intake.

378   By looking at changes in local and regional fish density and biomass patterns, we were able to

379   simultaneously detect asymmetrical increases in biomass of the surrounding food web along the

380   accessibility gradient. These changes in biomass suggest asymmetrical access to net-pen resources

381   may drive differential productivity in the surrounding food web (e.g., cold-water species elevated).

382   Investigation into patterns of fish density surrounding the net-pen showed increased density locally

383   around the net-pen, suggesting surrounding species aggregate to forage on the released net-pen

384   subsidy. These aggregations were also stronger at night, when fish are actively foraging. Our results

385   follow previous aquaculture literature that demonstrates increased fish densities surrounding fish farm

386   operations in both marine and freshwater systems (Ferndandez-Jover et al., 2007; Johnston et al.,

387   2010; Rennie et al., 2019) Investigation of the distribution of regional fish biomass demonstrated that

388   Parry Sound had a higher proportion of biomass of cold-water species relative to sites with no point-

389   source subsidy present. The high biomass of cold-water species was largely driven by higher

390   proportion of biomass of lake trout relative to all other sites studied in Georgian Bay, Lake Huron.

391   Parry Sound also supports a natural reproducing lake trout population that does not undergo yearly

392   stocking (UGLMU, 2018). South Bay was the only site that had comparable proportion of total

393   biomass in the cold-water guild. However, here the guild is dominated by whitefish and there are no

394   lake trout present, it also supports a lower diversity of cold-water fish than Parry Sound (Appendix 1:

395   Table S3). The significantly higher proportion of offshore biomass in Parry Sound, and presence of a

396   naturally reproducing off-shore top predator that strongly accesses net-pen feed, suggests net-pen

397   subsidies may be increasing off-shore productivity. Further research investigating how these released

398   subsidies are influencing basal food web productivity (i.e., zooplankton biomass) will be helpful in

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399   elucidating the mechanism through which net-pen subsidies are driving significantly higher cold-water

400   guild biomass in Parry Sound.

401   Through examination of trophic position of net-pen associated species and control species

402   throughout Lake Huron, we detected significant increases in trophic position of cold-water species in

403   Parry Sound relative to all other sites in Lake Huron, which was not reflected in cool and warm water

404   species. As higher trophic positions indicate longer food chains (Vander Zanden et al., 1999), these

405   results suggest that thermal guilds with the greatest accessibility to net-pen subsidies (in this case cold-

406   water guild) have a significantly longer food chain than guilds with less access. Therefore, the gradient

407   in subsidy accessibility drives asymmetric changes in surrounding food web structure (i.e., lengthening

408   of cold water food chain, no lengthening of cool-warm water food chain). These results are supported

409   by previous work conducted by Johnson et al. (2018) that provided the first evidence of higher trophic

410   position in cold-water species in net-pen associated sites. Asymmetrical changes in food web structure

411   can fundamentally rewire whole food webs (Bartley et al., 2019), which influences whole food web

412   stability and function in yet unknown ways. As accessibility is a main driver of these asymmetric

413   changes in surrounding food web that may influence whole food web stability, our research highlights

414   the importance of looking at subsidy accessibility when trying to understand or predict subsidy-driven

415   food web dynamics.

416   Here, we provide the first evidence that accessibility gradients to subsidies do exist and can

417   largely influence the strength of subsidy assimilation and changes in resultant food web productivity

418   and structure. To determine if subsidy accessibility is ubiquitous across ecosystems (i.e., is this a

419   characteristic of subsidies that is always relevant), more empirical studies investigating this concept

420   are needed. Additionally, theoretical work focused on understanding how asymmetrical changes in

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421   productivity and structure based on differential accessibility scenarios influence food web stability and

422   function are needed to understand the dynamical implications of subsidy accessibility.

423   Global change and increasing human populations are continually altering the flow of natural

424   and anthropogenic subsidies throughout ecosystems, thus it is imperative to understand how these

425   subsidies, in combination with global changes, will influence future food web stability and function.

426   Elucidating key subsidy characteristics that drive changes in recipient food web structure and function

427   is essential. Here, we contribute another key subsidy characteristic, subsidy accessibility, that has yet

428   to be considered in subsidy literature. While this study shows how accessibility is key in determining

429   food web responses to an anthropogenic subsidy, we argue this concept can be more widely applied to

430   all subsidies and is a key characteristic of subsidy-receiving food web interactions that must be

431   considered when trying to understand subsidy impacts on receiving ecosystem stability and function

432   under continued global change.

433   **Acknowledgments**

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535   **Figure 1.** Conceptual figure of food web interactions within (a) natural temperate lake food web

536   and (b) a temperate lake food web with a pelagic point-source anthropogenic subsidy input,

537   which here is released net-pen feed. It is predicted that net-pen feed will alter food web structure

538   through asymmetric accessibility to surrounding food web. Therefore, we expect to see strong

539   assimilation of net-pen feed in cold water fish and subsequent increases in trophic level and

540   biomass. It is expected that some assimilation occurs in cool water fish, and no assimilation of

541   net-pen feed occurs in warm water guild.

542   **Figure 2.** Principal component analysis of fatty acids >1% of total fatty acid composition of all prey

543   fish, baseline, and feed samples analyzed in (a) 2016 and (b) 2017. Feed separated from lake biota

544   (except for littoral baselines) along PC1 in both 2016 and 2017. 2016 feed was separated by 3 FA

545   (higher levels of 18.2n6, 18.1n9, 20.1n9). In 2017, feed was separated by 4 FA (higher levels of

546   18.2n6, 18:3n3, 18.1n9, and 16:1n7).

547   **Figure 3**. (i) PCA and (ii) univariate boxplot comparisons of feed indicator fatty acids (FIFA)

548   for (a) cool and (b) warm generalist, top predators and (c) cool water forage fish collected from

549   Parry Sound (PS) (anthropogenic subsidy input site) and control sites (Shawanaga Bay, SHN) in

550   2016.

551   **Figure 4.** (i) PCA and (ii) univariate boxplot comparisons of feed indicator fatty acids (FIFA)

552   for (a) cold, (c) cool, and (e) warm generalist top predators, and (b) cold and (d) cool water

553   forage fish collected from Parry Sound (PS) (anthropogenic subsidy input site) and control sites

554   (Dyers Bay, DB; Shawanaga Bay, SHN; Key Harbour, KH) in 2017.

555   **Figure 5.** Euclidean distance between centroids of net-pen and control sites, calculated

556   separately for each species and year, in relation to species' final temperature preference. Blue

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557   represents species in the cold thermal guild, yellow and orange are species in the cool thermal

558   guild, and red is species in the warm thermal guild.

559   **Figure 6**. Change in fish density (fish count/m^2) relative to distance from net-pen facility (m) in Parry

560   Sound for (a) day time transect and (b) night time transect in July 2017. Map inset shows transect

561   location in relation to net-pen facility in Parry Sound (red box), Georgian Bay. Fish density declined

562   with increasing distance from net-pen during the day, however this decline was not significant

563   (R^2=0.158, p = 0.0821). Fish density significantly declined with increasing distance from net-pen

564   facility at night (R^2 =0.43, p <0.0001).

565   **Figure 7**. Distribution of biomass proportion across thermal guilds of all species collected from

566   MNRF BSM sampling in Georgian Bay, Lake Huron in 2017. Thermal guilds are indicated by colour,

567   asterisks next to site name indicate sites with presence of net-pen facility. Parry Sound and South Bay,

568   both of which contain net-pen aquaculture facilities, demonstrate higher proportion of species biomass

569   in the cold thermal guild (off-shore species) relative to control sites. Specifically, cold water species

570   biomass in Parry Sound is 2.0 – 7.9 times higher than control sites sampled throughout Georgian Bay,

571   where no net-pen aquaculture facilities were located. High relative biomass of cold water species in

572   Parry Sound to control sites was not reflected in the cool and warm water guilds.

573   **Figure 8.** Trophic position of (a) cold, (b) cool and (c) warm generalist top predators, and (d) cold and

574   (e) cool water forage fish collected from Parry Sound (indicated in red) and control sites in 2017.

575   Samples are a combination of those collected by our research team and the MNRF. Asterisks indicate

576   significant differences based on Tukey post-hoc analysis.
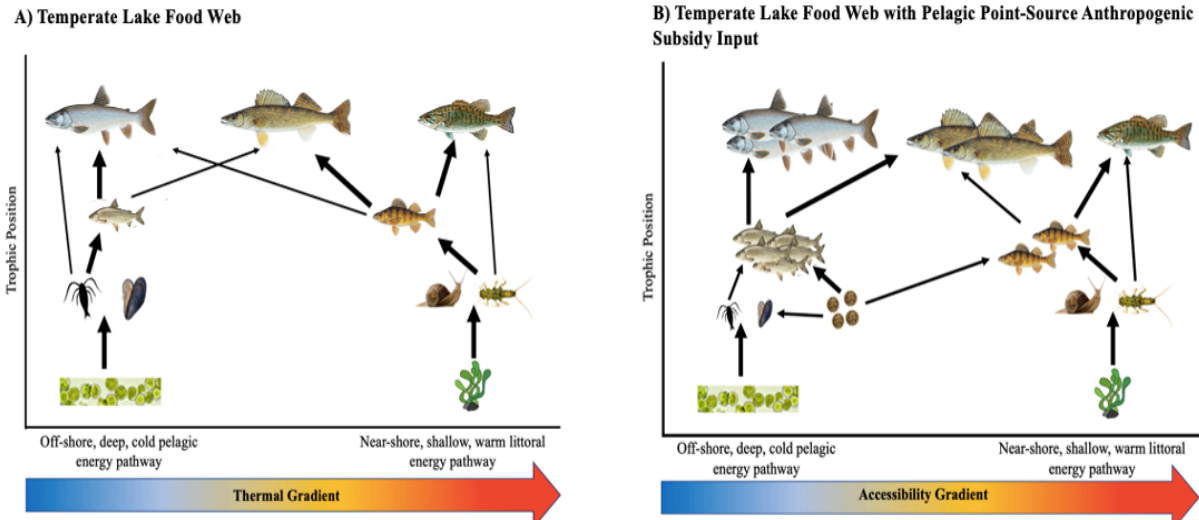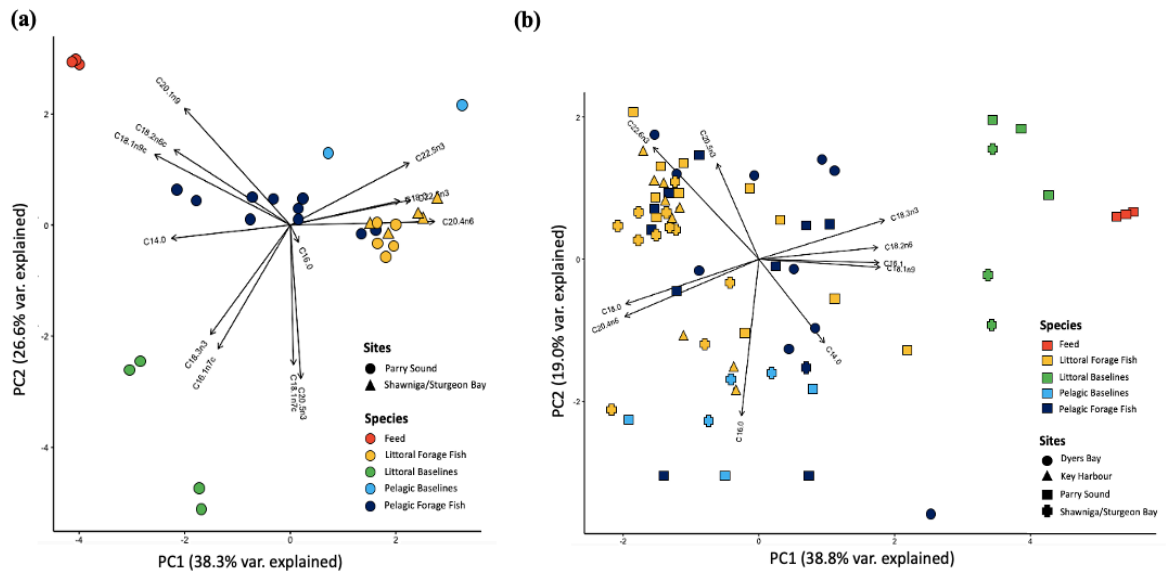
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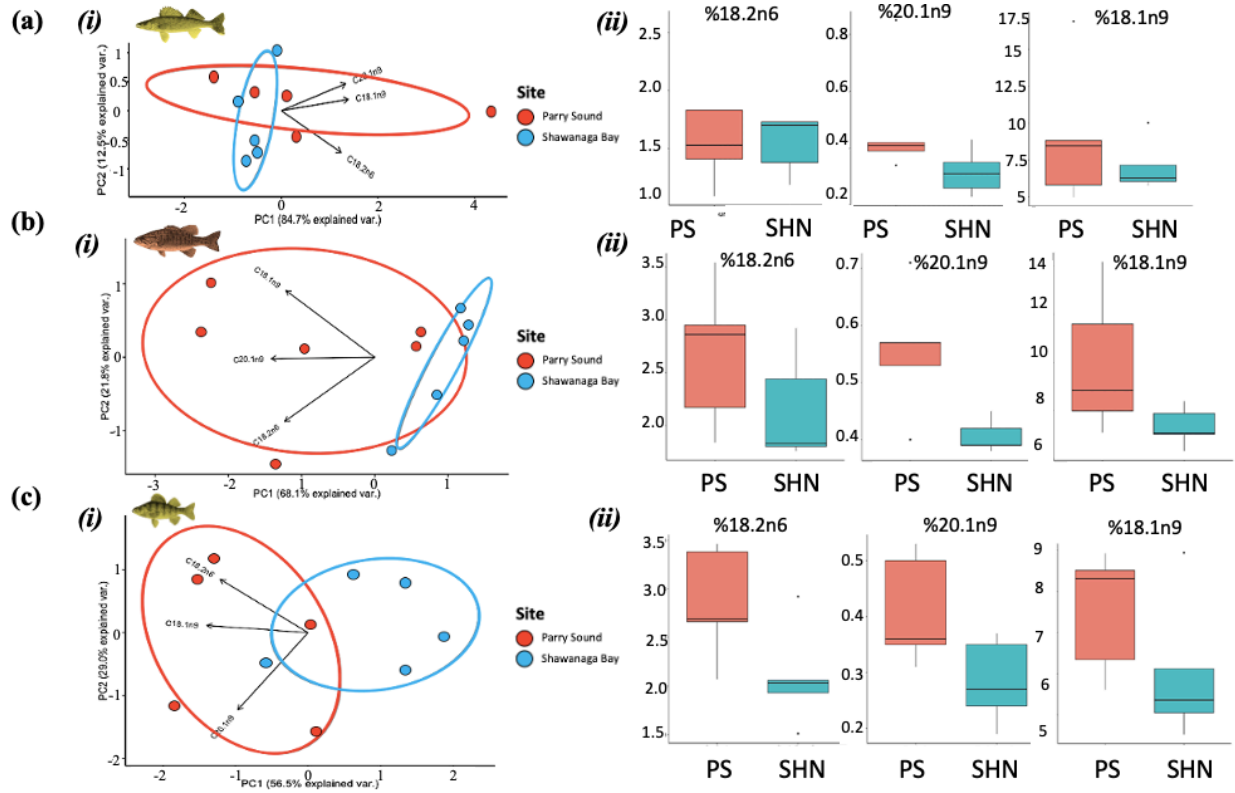
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580 **Figures**

581 **Figure 1**

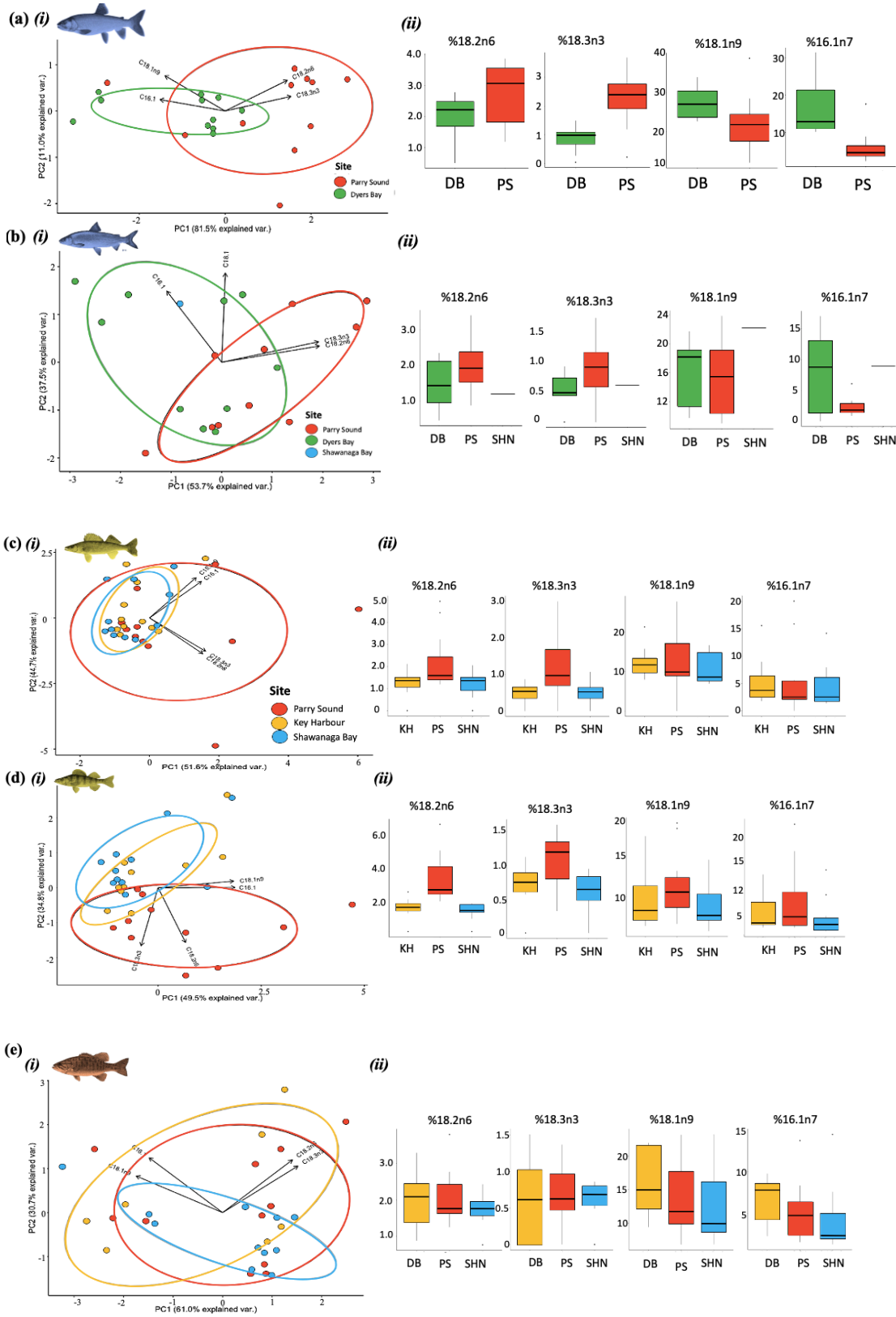
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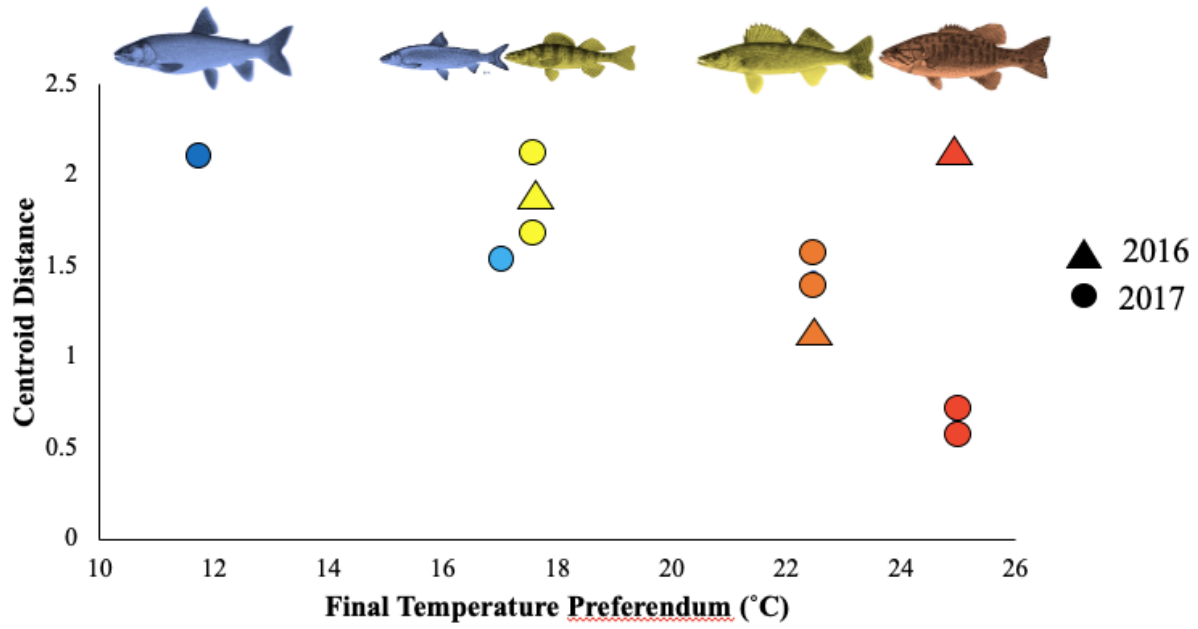584   **Figure 2**

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591 **Figure 4**
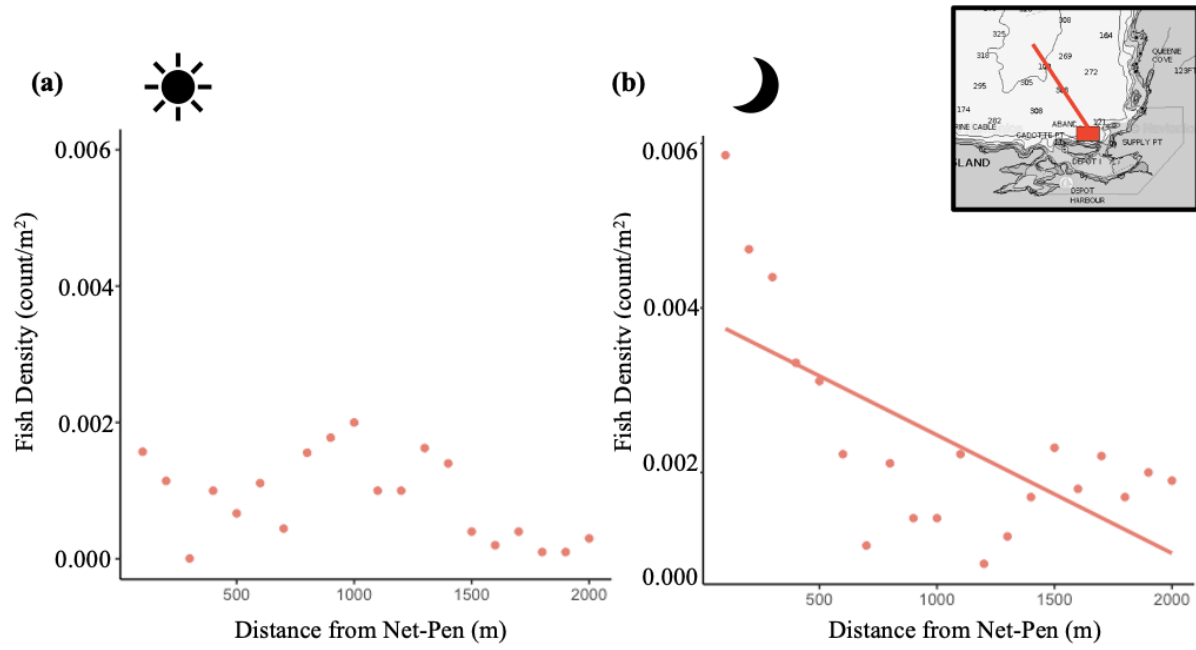

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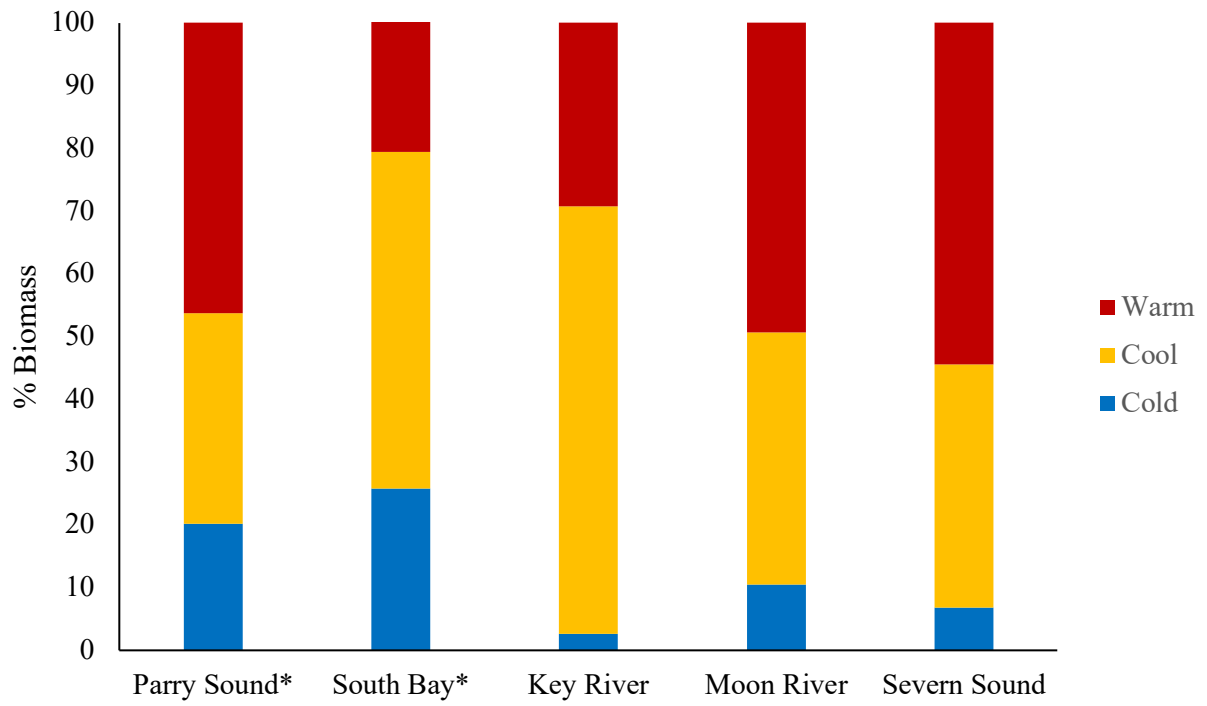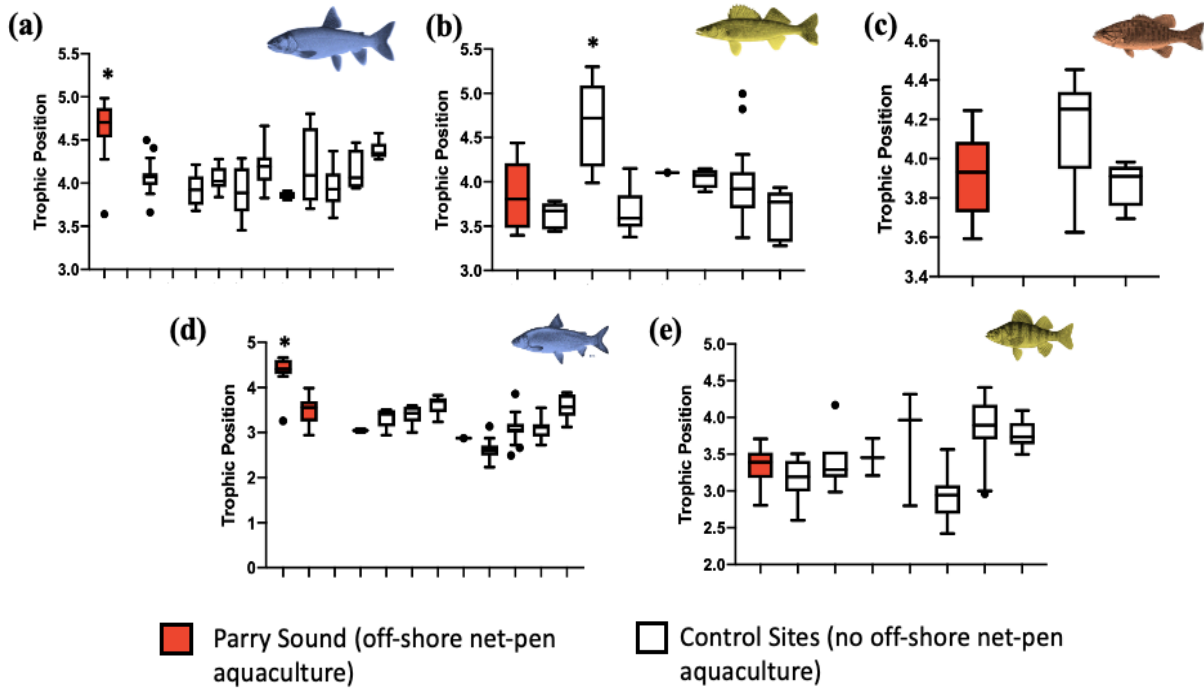595 **Figure 5**

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604 **Figure 8**

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