Not all jellyfish are equal: isotopic evidence for inter- and intraspecific variation in jellyfish trophic ecology

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Jellyfish are highly topical within studies of pelagic food-webs and there is a growing realisation that their role is more complex than once thought. Efforts being made to include jellyfish within fisheries and ecosystem models are an important step forward, but our present understanding of their underlying trophic ecology can lead to their oversimplification in these models. Gelatinous zooplankton represent a polyphyletic assemblage spanning >2,000 species that inhabit coastal seas to the deep-ocean and employ a wide variety of foraging strategies. Despite this diversity, many contemporary modelling approaches include jellyfish as a single functional group feeding at one or two trophic levels at most. Recent reviews have drawn attention to this issue and highlighted the need for improved communication between biologists and theoreticians if this problem is to be overcome. We used stable isotopes to investigate the trophic ecology of three co-occurring scyphozoan jellyfish species (*Aurelia aurita*, *Cyanea lamarckii* and *C. capillata*) within a temperate, coastal food-web in the NE Atlantic. Using information on individual size, time of year and $\delta^{13}C$ and $\delta^{15}N$ stable isotope values we examined: (1) whether all jellyfish could be considered as a single functional group, or showed distinct inter-specific differences in trophic ecology; (2) Were size-based shifts in trophic position, found previously in A. aurita, a common trait across species?; (3) When considered collectively, did the trophic position of three sympatric species remain constant over time? Differences in δ15N (trophic position) were evident between all three species, with size-based and temporal shifts in δ15N apparent in *A. aurita* and *C. capillata*. The isotopic niche width for all species combined increased throughout the season, reflecting temporal shifts in trophic position and seasonal succession in these gelatinous species. Taken together, these findings support previous assertions that jellyfish require more robust inclusion in marine fisheries or ecosystem models.

1   Not all jellyfish are equal: isotopic evidence for inter- and intraspecific variation in jellyfish

2   trophic ecology

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20 **Introduction**

21

22 Jellyfish (here considered as Phylum Cnidaria; Class Scyphozoa) are a conspicuous, yet long-

23 overlooked component of pelagic marine systems. In recent years the notion of gelatinous

24 species as merely carbon sinks, or trophic dead ends has become largely obsolete (Arai 2005;

25 Hansson & Norrman 1995) and there is renewed interest in their trophic ecology (Stoner &

26 Layman 2015; Sweetman et al. 2014). Beyond widely-recognised obligate predators of jellyfish

27 such as leatherback turtles (Houghton et al. 2006), Arai (2005) drew attention to a wide range of

28 opportunistic carnivores such as molluscs, arthropods, reptiles and birds that feed upon gelata

29 episodically. More recently, opportunist scavenging on jellyfish has been observed in the deep-

30 sea (Sweetman et al. 2014) as well shallower benthic environments (Stoner & Layman 2015).

31 From a perspective of top-down control, it is also known that the collective prey-consumption

32 rates of gelatinous aggregations can be so high that predation can directly or indirectly control

33 the population size of other zooplanktonic organisms including larval fish (Nielsen et al. 1997;

34 Purcell 1992). Moreover, evidence of sized-based trophic shifts in the moon jellyfish *Aurelia*

35 *aurita* (Linnaeus, 1758) (Fleming et al. 2011; Graham & Kroutil 2001) suggest that jellyfish

36 could themselves exhibit size-associated shifts in trophic ecology, *e.g.* similar to those shown by

37 fishes (Graham et al. 2007).

38

39 Prompted by a growing body of evidence, Pauly *et al*. (2009) stressed that the functional role of

40 gelatinous taxa requires more robust inclusion in marine fisheries or ecosystem models. At

41 present, such species are typically considered as a single functional group or an 'average' group

42 of animals, feeding on the same prey throughout their life history (Boero et al. 2008; Pauly et al.

43 2009). Indeed, out of 100 models considered, only 23 % incorporated jellyfish as a distinct

44 functional group (normally feeding at a single trophic level) and only 4 % of models considered

45 them in any greater detail, e.g. feeding at two trophic levels (Pauly et al. 2009). Consequently,

46 seasonal or ontogenetic shifts in diet (Fleming et al. 2011; Graham & Kroutil 2001), intra-

47 specific differences in prey types (Fancett 1988)) and intra-guild predation (Bayha et al. 2012;

48 Robison 2004; Titelman et al. 2007) are typically over-simplified or disregarded entirely. Pauly

49 et al. (2009) and Doyle et al. (2014) have made a number of suggestions for researchers working

50 with gelatinous species on how to generate data that are useful to theoreticians. These studies

51 highlight the fact that the ecological-modelling community cannot be expected to consider

52 jellyfish in adequate detail, if the data required are not provided by other researchers (Doyle et al.

53 2014). This is a valid point, but until recently many questions surrounding the trophodynamics of

54 gelatinous species appeared intractable, given the spatial and temporal variability of aggregations

55 (Doyle et al. 2007; Houghton et al. 2007), the broad-scale over which they can occur (Doyle et

56 al. 2008) and methodological limitations (Purcell 2009).

57

58 Within this broad context, the aim of this study was to examine trophic variation in three

59 sympatric jellyfish species (*Aurelia aurita* (Linnaeus, 1758), *Cyanea lamarckii* (Péron &

60 Lesueur, 1810) and *C. capillata* (Linnaeus, 1758)) in a temperate coastal marine system.

61 Strangford Lough in Northern Ireland was identified as an ideal study system as it supports an

62 annual succession of gelatinous zooplankton species from early May to late August (Fleming et

63 al. 2013). We used stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to consider size-based and temporal shifts in

64 the trophic ecology of the three jellyfish species, both individually and collectively as a dominant

65 large gelatinous zooplankton community. Isotopic approaches have been used widely to examine

66 the trophic ecology of marine and estuarine systems in general (Peterson & Fry 1987), and are

67 gathering momentum for the study of gelatinous species (Kogovšek et al. 2014; Nagata et al.

68 2015; Pitt et al. 2008). To provide data that might aid the further inclusion of jellyfish into

69 ecosystem models, our analyses were aligned to examine three specific questions: (1) could all

70 jellyfish be considered as a single functional group or was there evidence for distinct inter-

71 specific differences in trophic ecology?; (2) were size-based shifts in trophic ecology found

72 previously in *A. aurita* a common trait across species?; and (3) when considered collectively, did

73 the trophic position and isotopic niche of three sympatric species remain constant over time?

74

75 **MATERIALS & METHODS**

76 **Collection and processing**

77 Strangford Lough (54° 28' 20.98"N 5° 35' 10.60"W; Northern Ireland) is a large, semi-enclosed

78 coastal embayment (150 km^2) that flows into the northern Irish Sea (see Maloy et al. 2013 for a

79 description). Three scyphozoan jellyfish species are persistently present in the lough but their

80 relative abundance varies over time. In May, the community is typically dominated by *Aurelia*

81 *aurita*, with an increase in *Cyanea lamarckii* in early June and *Cyanea capillata* in July (Fleming

82 et al. 2014). All three species disappear from the water column in the same order from late July

83 onwards (Fleming et al. 2014; Fleming et al. 2013). Medusae of these three jellyfish species were

84 sampled monthly from Strangford Lough (May 2010 to September 2010). Jellyfish were

85 collected near the surface from a small boat using a dip net (mesh size 1 mm) for smaller

86 jellyfish and a larger net (5 mm mesh size) for larger individuals. Sampling was conducted in a

87 non-random manner, as our aim was to collect sufficient individuals to allow for balanced

88 statistical comparisons (e.g. across months). Unfortunately, owing to temporal variation in the

89   abundance of the different species, and often challenging weather conditions, it was not possible

90   to ensure a balanced number of samples per species.

91

92   Filter-feeding bivalves (*Mytilus* spp.) and grazing gastropods (*Littorina saxatilis* (Olivi)) were

93   sampled over the study period from intertidal areas adjacent to the jellyfish sampling sites over

94   the same period (Woodland et al. 2012). These species are long-lived, dominant and ubiquitous,

95   providing a measure of isotopic baselines of the pelagic (bivalve) and benthic (gastropods)

96   primary production pathways as suggested by Post (2002) and supported by others (e.g. Mallela

97   & Harrod 2008; Richoux & Ndhlovu 2014). Furthermore, isotopic turnover rates (expressed as

98   half-life) in the moon jellyfish (*Aurelia aurita*) recently described by D'Ambra et al. (2014) who

99   estimated a half-life for $\delta^{13}C$ (10.8 days) and $\delta^{15}N$ (9.7 days) are similar to that of *Mytilus* ($\delta^{13}C$

100   = 9 days; $\delta^{15}N$ = 14 days) (Dubois et al. 2007), suggesting a similar ability to track temporal

101   shifts in baseline isotope values.

102

103   **Laboratory and SIA analysis**

104   All jellyfish samples were collected and processed immediately to prevent potentially marked

105   effects of freezing and ethanol preservation (Fleming et al. 2011). *A. aurita, C. lamarckii* and *C.*

106   *capillata* were weighed and measured (wet mass: ± 1g; bell diameter: ± 1cm), then medusae

107   were rinsed thoroughly in filtered seawater, after which bell (mesoglea) tissues were separated

108   and dried at 60°C in a drying oven following Fleming et al. (2011). Samples were ground to a

109   fine powder in an agate mortar and pestle and then weighed into tin cups prior to stable isotope

110   analysis. Preliminary analyses revealed that optimal sample mass for mass spectrometry varied

111   between taxa *i.e. A. aurita* ≈ 12 mg; *C. lamarckii* ≈ 2.4 mg, *C. capillata* ≈ 5.1 mg and other taxa

112   $\approx$ 0.8 mg). Samples were analysed for $\delta^{13}C$, $\delta^{15}N$ and C:N at the East Kilbride Node of the

113   Natural Environment Research Council Life Sciences Mass Spectrometry Facility via continuous

114   flow isotope ratio mass spectrometry using an ECS 4010 elemental analyser (Costech, Milan,

115   Italy) interfaced with a Delta XP mass spectrometer (Thermo Electron, Bremen, Germany). The

116   standard deviation of multiple analyses of an internal gelatine standard was ~ 0.1 ‰ for both

117   $\delta^{13}C$ and $\delta^{15}N$.

118

119   **Statistical analysis**

120   Prior to analysis, bell mass, bell diameter and stable isotope data were $\log_{10}$-transformed to

121   improve normality and reduce heteroscedasticity ($\delta^{13}C$ data were $\log_{10}+40$ transformed due to

122   their negative values). Recently evidence has emerged that air-drying gelatinous tissue can result

123   in ^{15}N enrichment in more proteinaceous species (Kogovšek et al. 2014). C:N ratios of the three

124   species were compared and found not to differ ($F_{2, 120} =$ 1.48, P = 0.232), suggesting that any

125   effect of air-drying would be consistent across species. We used various statistical approaches to

126   characterise and compare the trophic ecology (inter-specific, intra-specific and community) of

127   the jellyfish species.

128

129   Permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001; Anderson et

130   al. 2008) in PRIMER 6.1.12 (Clarke & Gorley 2006; Clarke & Warwick 2001) was used to

131   examine variation in the location of centroids of $\log_{10}$ transformed $\delta^{15}N$-$\delta^{13}C$ data, based on a

132   Euclidean similarity matrix ($n_{permutations}$ = 9 999). PERMANOVA was used to examine variation

133   in bell $\delta^{15}N$ and $\delta^{13}C$ values by species (inter-specific variation) and sample month (intra-

134   specific variation). Here, it is assumed that where $\delta^{15}N$-$\delta^{13}C$ centroids overlap (i.e. are not

135   significantly different), then trophic ecology is similar e.g. between species or month. As some

136   small (n ≤ 3) sample sizes were recorded for species across the different months (*C. capillata* in

137   May; *A. aurita* and *C. lamarckii* in August), it was not possible to make a balanced two-way

138   analysis for the entire study period. A full two-way PERMANOVA examining isotopic variation

139   associated with Species and Month (and the Species x Month interaction) was conducted for

140   June and July only. One-way PERMANOVA was used to compare variation within species

141   across months.

142

143   Two-way PERMANOVA was used to examine whether $\delta^{15}N$-$\delta^{13}C$ values from baseline

144   indicators associated with the pelagic and benthic pathways varied either between functional

145   groups or over time (survey month). We also conducted a similar univariate two-way

146   PERMANOVA comparing temporal shifts in $\delta^{15}N$ data from the two functional groups in order

147   to examine whether shifts in jellyfish $\delta^{15}N$ were related to changes at the base of the food web or

148   in apparent jellyfish trophic level.

149

150   As jellyfish are often considered as a single functional group, we examined how an indicator of

151   community level trophic position varied across the survey period by pooling $\delta^{15}N$ data from all

152   three jellyfish species and conducting a univariate PERMANOVA with month as a fixed

153   independent factor.

154

155   We used the SIBER procedure (Stable Isotope Bayesian Ellipses in R) within the R package

156   SIAR (Jackson et al. 2011) to examine variation in jellyfish isotopic niche space. This approach

157   relies on the concept that multiple stable isotope ratios measured from consumers represent niche

158 dimensions, *e.g.* variation in δ^{13}C reflects use of different energy sources, or habitats, while δ^{15}N

159 provides information on the trophic level at which a consumer feeds (Peterson & Fry 1987). This

160 so called 'isotopic niche' or 'δ-space' (Newsome et al. 2007) is thought to reflect the trophic

161 niche of groups of consumers (Bearhop et al. 2004; Fink et al. 2012; Layman et al. 2007), where

162 more isotopic variation reflects a larger consumer isotopic niche, assuming that spatial or

163 temporal variation in baseline isotopic values is considered. Here we use Bayesian Standard Area

164 Ellipses (SEA$_B$), as the use of Bayesian inference allows the incorporation of uncertainty such as

165 small sample sizes (Jackson et al. 2011). This iterative approach uses Monte Carlo Markov-

166 Chain simulation to construct ellipses characterising isotopic variation that provide a robust

167 indicator of isotopic niche width. We used this technique to characterise temporal variation in the

168 trophic niche of the three jellyfish species, as well as overlap between species. We also examined

169 temporal variation in SEA$_B$ values calculated for the jellyfish community as a whole (i.e. all

170 three species of jellyfish combined). In order to examine the differences in isotopic niche area

171 (SEA$_B$) between different consumer groups, we calculated probabilities from posterior

172 distributions (based on 100,000 draws) of the parameters of model M given the prior data D

173 (Pr($M|D$)). These maximum likelihood comparisons provide direct probabilities of differences

174 rather than the traditional frequentist test of a null-hypothesis. In order to differentiate these

175 comparisons, maximum-likelihood based probabilities are reported here as percentages.

176

177 In stable isotope studies, consumer trophic position is typically estimated from δ^{15}N data, which

178 are corrected for baseline variation and trophic fractionation (Post 2002). Although we had

179 reliable data on pelagic and benthic δ^{15}N baselines (see above), information on jellyfish trophic

180 enrichment factors (TEFs) is extremely limited. D'Ambra et al. (2014) recently provided TEFS

181   for *A. aurita*, in what represents the only experimental estimate of jellyfish trophic fractionation

182   in the literature. The mean ± SD TEFs estimated by D'Ambra et al. for *A. aurita* ($\Delta^{13}C = 4.3 \pm$

183    $0.2‰$; $\Delta^{15}N = 0.1 \pm 0.2‰$) are very unusual and contrast markedly with the average TEFS more

184   commonly seen in the literature (e.g. Post (2002): $\Delta^{13}C = 0.4 \pm 1.3‰$; $\Delta^{15}N = 3.4 \pm 1‰$;

185   McCutchan et al. (2003) ($\Delta^{13}C = 0.5 \pm 1.3‰$, $\Delta^{15}N = 2.3 \pm 1.5‰$). As use of the jellyfish specific

186   TEFs provided by D'Ambra (2014) resulted in unfeasibly high trophic positions for the jellyfish

187   species, including *A. aurita*, we did not make direct estimates of trophic position, but provide

188   indirect estimates by presenting $\delta^{15}N$ data.

189

190   Finally normal linear least-squares regression was used to examine how $\log_{10}$ transformed stable

191   isotope values ($\delta^{13}C$ data were $\log_{10}+40$ transformed) varied with individual size (bell wet mass

192   and diameter).

193

194   Statistical analyses were conducted using routines in PRIMER-E 6 (Clarke & Gorley 2006) and

195   SYSTAT 13.1 (SYSTAT Software Inc 2009). SIBER analyses (Jackson et al. 2011) were

196   conducted using SIAR (Parnell et al. 2010) in R version 3.1.2 (R Development Core Team

197   2014). An alpha level of 0.05 is used throughout to indicate statistical significance.

198

199 **RESULTS**

200 **Baseline variation**

201 Comparisons of baseline indicator (filter feeding and grazing molluscs) $\delta^{15}N$-$\delta^{13}C$ values across

202 the study period using two-way PERMANOVA showed strong evidence of isotopic differences

203 between the two functional groups (Pseudo-$F_{1,108}$ = 82.44, P = 0.0001), but less evidence for

204 marked temporal differences (Month: Pseudo-$F_{2,108}$ = 2.64, P = 0.06). There was no evidence for

205 a significant interaction between these two factors (Pseudo-$F_{2,108}$ = 0.04, P = 0.99), indicating

206 that the isotopic differences between the two functional groups were maintained over time.

207

208 We also examined $\delta^{15}N$ values from filter feeding and grazing molluscs as they provide a

209 reference for measurements of consumer trophic position relative to the base of the food web.

210 Baseline $\delta^{15}N$ values differed between the two functional groups (Pseudo-$F_{1,108}$ = 59.57, P =

211 0.0001) with benthic grazers (mean ± SD $\delta^{15}N$ = 11.2 ± 1.08, n = 58) being ^{15}N enriched by 1.5

212 ‰ relative to filter feeding bivalves (bivalve = 9.7 ± 0.7, n = 56) but were consistent across the

213 study period (PERMANOVA on $\log_{10}$-transformed $\delta^{15}N$ data; Month: Pseudo-$F_{2,108}$ = 0.48, P =

214 0.725). The lack of an interaction between the two factors (Month x Functional Group: Pseudo-

215 $F_{2,108}$ = 0.087, P = 0.91) indicated that the differences in $\delta^{15}N$ between the two functional groups

216 remained constant over time.

217

218

219 **Inter-specific variation**

220 A total of 122 medusae were collected from the surface of the water column comprising *Aurelia*

221 *aurita* (*n* = 43), *Cyanea lamarckii* (*n* = 36) and *C. capillata* (*n* = 43). Data collected across the

222   entire study for the three jellyfish species (Fig. 1) showed considerable intraspecific variation

223   and apparent isotopic overlap between the species. However, when $\delta^{15}N$ and $\delta^{13}C$ data for

224   individual species were compared over time, differences became apparent (Table 1; Fig. 2).

225

226   A full two-way PERMANOVA comparing the influence of survey month and species was only

227   possible for all three species in the months of June and July when medusae of all species were

228   present. The analysis of log_{10}-transformed data revealed that $\delta^{15}N$-$\delta^{13}C$ centroid location varied

229   significantly between the three jellyfish species (Pseudo-$F_{2,71}$ = 5.01, P = 0.006) and survey

230   month (Pseudo-$F_{1,71}$ = 5.1, P = 0.02). However, there was no interaction between species and

231   survey month ($F_{2,71}$ = 0.25, P = 0.82) indicating that temporal shifts in $\delta^{13}C$-$\delta^{15}N$ isotope values

232   were similar across the three scyphozoan species in June and July. Pairwise comparisons showed

233   that *A. aurita* were isotopically distinct from both *Cyanea* species in June (*C. lamarckii* P $\leq$

234   0.0043; *C. capillata* P = 0.02), and from *C. lamarckii* in July (P = 0.03). The $\delta^{15}N$-$\delta^{13}C$ centroids

235   of the two *Cyanea* species overlapped during these months (June: P = 0.89; July: P = 0.43).

236

237   Next, we considered inter-specific differences in isotopic niche width (Fig. 3). Between-species

238   comparisons (data pooled from all months) showed that *C. capillata* had the largest mean (95 %

239   credibility limits) isotopic niche width of 6.90 (4.95 - 9.03) ‰^{2}, compared to *A. aurita* (4.94

240   (3.55 - 6.46) ‰^{2}) or *C. lamarckii* (5.49 (3.84 - 7.32) ‰^{2}). Maximum-likelihood pairwise

241   comparisons indicated a borderline probability (Probability (P) = 94 %) that across the entire

242   study the isotopic niche width of *C. capillata* was larger than that of *A. aurita*. There was no

243   statistical support (P = 85 %) for differences between *C. capillata*, and its congeneric *C.*

244 *lamarckii*. There was a 67 % probability of differences in isotopic niche width size between *A.*

245 *aurita* and *C. lamarckii*.

246

247 **Intra-specific variation**

248

249 Although *A. aurita* were captured in each of the survey months (Fig. 2), sufficient samples for

250 analysis were not recorded in August (n = 2), and statistical comparisons here are limited to the

251 period May-July (See Table 1 for sample sizes). During this period, the location of *A. aurita*

252 δ^{15}N-δ^{13}C centroids varied significantly (One-way PERMANOVA Pseudo-$F_{2,38}$ = 15.19, P =

253 0.0001), indicating that *A. aurita* underwent an isotopic shift over the study period. Pairwise tests

254 showed that δ^{15}N-δ^{13}C centroids shifted between May and both June (t = 4.49, P = 0.0002) and

255 July (t = 4.77, P = 0.0001). δ^{15}N-δ^{13}C values overlapped in June and July (t = 1.6, P = 0.12). The

256 difference between May and the other months reflected enrichment in ^{13}C and to a lesser degree

257 ^{15}N from May to the later months.

258

259 Sample sizes in *C. lamarckii* were relatively low throughout the study, with large numbers only

260 being encountered in June (Table 1). *C. lamarckii* showed significant temporal shifts in the

261 location of the δ^{15}N-δ^{13}C centroids (May – July: Pseudo-$F_{2,31}$ = 15.46, P = 0.0001). Pairwise tests

262 revealed that centroids differed between May and both June (t = 5.15, P = 0.0002) and July (t =

263 6.58, P = 0.001), but overlapped between June and July (t = 0.63, P = 0.56). Isotopically, *C.*

264 *lamarckii* became increasingly ^{13}C and ^{15}N enriched over the survey period (Fig. 2, Table 1).

265

266 Only two *C. capillata* were available for analysis in May, but in the following months, $\delta^{15}N$-$\delta^{13}C$

267 centroids for this species changed significantly (June-August: Pseudo-$F_{2,38}$ = 4.44, P = 0.008).

268 Pairwise tests indicated that this shift was relatively gradual, with isotopic overlap in June and

269 July (t = 1.87, P = 0.06) and July-August (t = 1.22, P = 0.22). Isotopic differences were most

270 marked at the extremes of the collection period: June – August (t = 2.79, P = 0.003).

271

272 Bayesian estimates of isotopic niche width (SEA$_B$) showed significant variation within species

273 during the study period (Table 3 & Fig. 3). Pairwise comparisons showed that *A. aurita* mean

274 isotopic niche width was lower in May relative to other months (Table 3, Fig. 3), with a 95 %

275 probability of a difference from June and a 98 % probability of a difference from July. The

276 isotopic niche width of *C. lamarckii* was reduced in May relative to June (P = 99 %) and July (P

277 = 96 %), but there were no obvious differences in isotopic niche width in June and July (P = 46

278 %). *C. capillata* was not recorded in sufficient numbers in May to allow analyses, but showed a

279 similar isotopic niche width through the June - August period (P range 50 – 60 %).

280

281 Both *A. aurita* and *C. capillata* showed positive linear relationships between log$_{10}$-transformed

282 $\delta^{13}C$ and wet mass ((Table 2, Fig. 4: *A. aurita* $F_{1,41}$ = 26.9, R^2 = 0.40, P < 0.001; *C. capillata* $F_{1,41}$

283 = 16.1, R^2 = 0.28, P < 0.001) and bell diameter (*A. aurita* $F_{1,41}$ = 26.3, R^2 = 0.39, P < 0.001; *C.*

284 *capillata* $F_{1,41}$ = 19.1, R^2 = 0.32, P < 0.001), indicating a shift in dietary source with size in these

285 species. However, there was no evidence for any such relationship in *C. lamarckii* for wet mass

286 ($F_{1,35}$ = 0.71, R^2 = 0.02, P = 0.405) or bell diameter ($F_{1,35}$ = 0.85, R^2 = 0.02, P = 0.363),

287 indicating that individuals of all sizes assimilated carbon from a similar range of sources. $\delta^{15}N$

288 increased with size (Fig. 4 & Table 2) in both *A. aurita* (log$_{10}$-transformed wet mass $F_{1,41}$ = 48.8,

289    $R^2 = 0.54$, $P < 0.001$; bell diameter $F_{1,41} = 46.2$, $R^2 = 0.53$, $P = <0.001$) and *C. capillata* (wet

290   mass $F_{1,41} = 22.1$, $R^2 = 0.35$, $P = <0.001$; bell diameter $F_{1,41} = 22.0$, $R^2 = 0.34$, $P < 0.001$). In all

291   cases, the slope of the $\log_{10}$-$\log_{10}$ relationship was < 1 (Table 2). As in the case of $\delta^{13}C$, *C.*

292   *lamarckii* showed no evidence of any size-based shift in $\delta^{15}N$ (wet mass = $F_{1,35} = 1.50$, $R^2 = 0.04$,

293    $P = 0.229$; bell diameter $F_{1,35} = 2.4$, $R^2 = 0.06$, $P = 0.131$).

294

295   **Variation at a whole community level**

296   As baseline $\delta^{15}N$ values were consistent over time (see 'Baseline variation' above), we were able

297   to use $\delta^{15}N$ as an indirect indicator of changes in whole community apparent trophic position

298   over time in the absence of reliable TEFs. $\delta^{15}N$ values for the dominant gelatinous zooplankton

299   community (All GZ) as measured here, varied over the study period (One-way univariate

300   PERMANOVA Pseudo-$F_{3,119} = 36.9$, $P = 0.0001$; Fig. 2), and showed relative increases in

301   apparent trophic position ($\delta^{15}N$) over time. Pairwise tests showed $\delta^{15}N$ in May was lower than in

302   all other months (June, $t = 6.2$, $P = 0.0001$; July, $t = 10.6$, $P = 0.0001$; August, $t = 13.3$, $P = $

303   0.0001). June $\delta^{15}N$ values were higher than May, but lower than subsequent months (May, $t = $

304   6.2, $P = 0.0001$; July, $t = 3.1$, $P = 0.0027$; August, $t = 4.4$, $P = 0.002$). There was no measurable

305   difference in whole community $\delta^{15}N$ values in July and August ($t = 1.9$, $P = 0.07$; Fig. 2).

306

307   We also examined temporal variation in the community isotopic niche width by pooling values

308   from the three jellyfish species (See all GZ values in Fig. 3). Mean (95 % credibility limits)

309   jellyfish isotopic niche width in May was lower than in June, July or August ($P = 100$ % in all

310   cases). However, isotopic niche for the combined jellyfish species began to change in position

311   and width as the season progressed with an increase in isotopic niche ($‰^2$ 95% credibility limits)

312   from May = 2.05 (1.31 – 2.89) to Aug = 5.72 (3.49 – 8.3), suggesting a broader trophic niche in

313   the latter months (P July > June = 54 %; P August > June = 76 %; P August > July = 70 %).

314

315

Discussion

Pauly et al. (2009) described jellyfish as arguably the most important predators in the sea. There is little ambiguity in this statement which, in part, prompted the present study. There is no doubt that the potential expansion of jellyfish in highly depleted oceans is a matter of grave concern (Lynam et al. 2006; Purcell et al. 2007), and an underlying knowledge of how jellyfish function within marine systems is required, so that long-standing trends in populations and communities can be teased apart from shifts in ecosystem structure. Stable isotope analysis offers a powerful biochemical approach to the estimation of trophic and dietary composition of individuals through to communities (Bearhop et al. 2004; Bolnick et al. 2003) and the results presented here support the idea that jellyfish play a more complex trophic role than once envisaged.

Consistency in baseline isotope values

Variation in $\delta^{15}N$-$\delta^{13}C$ values measured from baseline indicators of the pelagic (filter feeding bivalve) and benthic (grazing gastropod) energy pathways was driven by functional group rather than survey month. This indicates that any temporal differences observed in jellyfish isotope values and the measures derived from them (i.e. isotopic niche space), reflected changes in jellyfish diet over time rather than shifts at the base of the food web.

Inter-specific differences in trophic ecology

At the whole study level, isotopic differences were evident between the three jellyfish species in terms of $\delta^{15}N$ and $\delta^{13}C$, with post-hoc comparisons highlighting differences between *A. aurita* and both *Cyanea* species in June, and with *C. lamarckii* in July. Conversely, the Cyanea species showed isotopic overlap during June and July. Comparisons of isotopic niche width showed that

339   differences were most marked between *A. aurita* v *C. capillata.* Taken together, these results

340   suggest differences in jellyfish behaviour and their capacity to capture and ingest a range of prey

341   items between these two genera (Figs 2 & 3).

342

343   Typically, scyphozoan jellyfish encounter rather than detect and pursue prey and use both

344   'passive ambush' and 'feeding current' feeding strategies with direct interception and filtering

345   through tentacles being used in both cases (Kiørboe 2011). Feeding currents are generated by

346   pulsation of the bell which varies in shape and size between species, with slower velocities

347   normally associated with smaller individuals (Costello & Colin 1994; Costello & Colin 1995;

348   Kiørboe 2011). Depending on the escape velocities of putative prey, differences in feeding

349   current velocity between different jellyfish species might lead to different prey being captured

350   and ingested; however, further work is required to link trophic position with morphological

351   characteristics in an empirical manner.

352

353   *A. aurita* have a much reduced capture surface (shorter tentacles) compared with the *Cyanea* spp.

354   Heeger & Möller (1987) found that the majority of prey capture by *A. aurita* in Kiel Harbour, N

355   Germany, occurred on the tentacles as opposed to the subumbrellar surface, so this reduced

356   capture area may account for the low trophic position and narrowest niche width of this species

357   in the present study.

358

359   Although they differ in terms of maximum individual size, the congenerics *C. lamarckii* and *C.*

360   *capillata* have similarities in both nematocyst complement (Ostman & Hydman 1997; Shostak

361   1995) and morphology (Holst & Laakmann 2013). Previous studies have reported predation of

362   *C. capillata* on *A. aurita* medusae, therefore it is possible that the differences observed with *A.*

363   *aurita* may be a symptom of intra-guild predation by the larger *C. capillata* (e.g. Hansson 1997;

364   Purcell 2003; Titelman et al. 2007).

365

366   The isotopic variation found in this study suggests niche partitioning and represents a host of

367   differences in morphology, bell pulsation strength, prey capture techniques and nematocyst

368   composition that enable differential prey capture (Bayha & Dawson 2010; Costello & Colin

369   1994; Peach & Pitt 2005). Therefore, caution must clearly be taken to avoid over-simplification

370   of jellyfish in ecosystem models. In a broader context, as gelatinous zooplankton span > 2,000

371   species (Condon et al. 2012), occupying habitats ranging from the deep ocean through to shallow

372   water near-shore environments, the inclusion of an 'average' jellyfish in such models is likely to

373   underestimate the collective impact in terms of energy flow or consumption of prey (Pauly et al.

374   2009).

375

376   **Intra-specific differences in trophic ecology**

377   *A. aurita* and *C. capillata* shifted their use of both energy source (δ^{13}C) and trophic position

378   (δ^{15}N) with increasing body size, independent of time (Fig. 4). This suggests that different sized

379   jellyfish medusae, present in the water column at the same time and with access to the same prey

380   field, feed at different positions in the food web (Fleming et al. 2011; Graham & Kroutil 2001).

381   The simultaneous presence of different sized medusae appears to be a consistent trait across a

382   range of species at temperate latitudes (Houghton et al. 2007), suggesting that jellyfish

383   reproductive cohorts are often poorly defined with a marked overlap within given seasons. *C.*

384   *lamarckii,* however did not exhibit a size-based shift in trophic position with increasing body

385   size. This most likely reflects the comparatively narrow size range of the medusae sampled (3.5 -

386   20 cm), with the species rarely exceeding a bell diameter of 30 cm (Russell 1970). By

387   comparison, *C. capillata* medusae spanned a far broader size range (6 - 85 cm) allowing size

388   related shifts in diet to be more easily identified. There are also size related differences in

389   toxicity; although *C. lamarckii* is as venomous as *C. capillata* (Helmholz et al. 2007), as both

390   species increase in size, so too do the size of their nematocysts (Ostman & Hydman 1997). These

391   findings suggest that body size in jellyfish may, to some extent, underpin their capacity to feed at

392   multiple trophic levels through ontogeny. There are some clear exceptions to this rule e.g. small

393   gelatinous species (< 12 cm bell diameter) such as box jellyfish *Chironex fleckeri* and *Carukia*

394   *barnesi* have extraordinarily powerful stings that enable them to capture relatively large prey

395   such as larval and small fishes (Carrette et al. 2002; Kintner et al. 2005; Underwood & Seymour

396   2007).

397

398   **The trophic position of the jellyfish community over time**

399   When considered as a whole, the $\delta^{15}N$ values of the scyphozoan jellyfish community in

400   Strangford Lough increased as the season progressed (Fig. 2), even though baseline levels

401   remained constant. This increase in $\delta^{15}N$ was unlikely to be a result of a general increase in size

402   of jellyfish over time, as a range of sizes of each species were collected and analysed each month

403   (see Appendix S1). Given that $\delta^{15}N$ baselines were constant across the study period, this

404   indicates that trophic position increased over time. In terms of isotopic niche width, there was an

405   interesting dissimilarity between the start of the season (May) and the following months (June,

406   July and August), suggesting a shift to a broader dietary niche in the latter months (Fig. 3). This

407   increased resource utilisation is consistent with previous studies that suggested jellyfish dietary

408   niches are extremely broad, with species operating as generalists (Dawson & Martin 2001; Ishii

409   & Båmstedt 1998; Schneider & Behrends 1998) feeding opportunistically across a range of

410   plankton (Båmstedt et al. 1997; Titelman et al. 2007). Therefore, our data suggest that a different

411   and possibly constrained resource pool is being exploited at the beginning of the 'jellyfish

412   season'. There are of course environmental factors such as temperature which could have an

413   effect on N metabolism & excretion in jellyfish (Morand et al. 1987; Nemazie et al. 1993) and

414   temperature can have a significant effect on isotopic turnover times in a range of taxa (see

415   Thomas & Crowther 2015). The temperature increase in Strangford Lough over the course of the

416   study was modest (from $8.7 - 14.2°C$) but cannot be discounted as a possible influence on

417   isotopic variation over time. The sequential change in species composition seen in Strangford

418   Lough could, in part, be the result of intra-guild predation (Bayha et al. 2012; Robison 2004;

419   Titelman et al. 2007), which may also contribute to the observed broadening in isotopic niche.

420   Additionally, the collective increase in trophic position over time may reflect species succession

421   in the lough with a general shift from an *A. aurita* dominated in system in May through to a *C.*

422   *capillata* dominated system in August (Fleming et al. 2014). Most likely our results reflect

423   interplay of these two scenarios but highlight the problems associated with assuming that

424   different jellyfish species occupy a single trophic position or ecological niche (Boero et al. 2008;

425   Pauly et al. 2009).

426

427   Interspecific and temporal variation in consumer isotopes values can be put into deeper

428   ecological context through the use of models to estimate trophic position (Post 2002) and

429   consumption patterns (Phillips et al. 2014). However, the use of these models requires reliable

430   estimates of trophic enrichment factors. We welcome the recent TEF estimates made by

431   D'Ambra et al. (2014) for *Aurelia* sp., however, we found that the use of their TEFS resulted in

432   unfeasibly high trophic positions for the *Aurelia* and other jellyfish in our system. For example,

433   using Post's (2002) basic model for tropic position resulted in a mean jellyfish trophic position

434   of 17, with the baseline provided by our mean *Mytilus* δ^{15}N values. As such, realistic estimates of

435   jellyfish trophic level and consumption made using tools requiring accurate TEFS (e.g. mixing

436   models) remain problematic. We therefore call for more experimental work to characterise

437   jellyfish TEFs.

438

439   **Conclusions**

440   All species showed temporal shifts in their location in δ^{15}N-δ^{13}C space across the study. Given

441   the lack of marked changes at the base of the food web, this suggests that the three jellyfish

442   species consumed different prey across the study period. Size-based shifts in δ^{13}C and δ^{15}N

443   values were evident in two of the three jellyfish species examined here, leading to an inference

444   that variation in body size in some way drives variation in the trophic ecology of a particular

445   species. Distinct differences in δ^{13}C and δ^{15}N values were found within and between species,

446   with evidence of niche segregation between *A. aurita* and the two *Cyanea* species. Niche width

447   for all species combined increased considerably throughout the season, reflecting interplay of

448   possible intra-guild predation, temporal shifts in δ^{13}C and δ^{15}N values and the seasonal

449   succession in gelatinous species.

450

451   Taken together, these lines of evidence reinforce the idea that scyphozoan jellyfish require more

452   elegant inclusion in ecosystem or fisheries-based models. The salient point here is that jellyfish

453   should not be averaged or defined as a single amorphous group with little reference to temporal

454   and allometric shifts in individual species or gelatinous communities alike.

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457

461

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647

648
649   **Figure captions**
650

651   Figure 1: Variation in $\delta^{13}C$ and $\delta^{15}N$ shown in three species of jellyfish over the whole study
652   period. (See Table 1 for summary statistics).

653
654   Figure 2: Box-whisker plots showing variation in $\delta^{13}C$ (upper panel) and $\delta^{15}N$ (lower panel) in
655   the three jellyfish species, and within the dominant GZ community (GZ; all three species
656   combined) over the study period. See Table 1 for sample sizes and other summary statistics. NB:
657   Baseline $\delta^{15}N$ values remained constant over this period, indicating that the increase in $\delta^{15}N$
658   values reflected a shift in trophic position rather than seasonal shifts at the base of the foodweb.
659   Boxes show inter-quartile range, and the bold horizontal bar indicates the median value.
660   Whiskers reflect values 1.5 x the interquartile range.
661
662   Figure 3: Variation in isotopic niche width (SEAB) between species (A. a = *A. aurita*; C. l = *C.*
663   *lamarckii*; C. c = *C. capillata*)) and within the dominant GZ community (GZ; all three species
664   combined) sampled over the survey period. Boxes represent the 50, 75 and 95 % Bayesian
665   credibility intervals estimated from 100,000 draws. Samples marked with *included less than 10
666   individuals (see Parnell et al. 2010). See Table 3 for statistical comparisons.
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668   Figure 4: Variation in bell $\delta^{13}C$ (A & B) and $\delta^{15}N$ (C & D) with bell diameter (A & C) and wet
669   mass (B & D). Note use of logarithmic scale on x-axes.

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Table 1**(on next page)**

Summary statistics

Table 1: Summary statistics for bell stable isotope and C:N ratios.

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Species	n	$\delta^{13}C$ (± SD) ‰	$\delta^{15}N$ (± SD) ‰	C:N (± SD)
Aurelia aurita **May**	16	-20.3 (0.5)	8.5 (1.1)	3.8 (0.1)
Aurelia aurita **June**	18	-18.2 (0.5)	10.3 (1.5)	3.5 (0.4)
Aurelia aurita **July**	9	-18.1 (0.7)	11.5 (1.5)	3.5 (0.4)
Aurelia aurita **August**	2	-17.3 (0.1)	11.8 (1.7)	3.7 (0.1)
Overall mean *A. aurita*	43	-19.0 (1.2)	9.7 (1.6)	3.6 (0.2)
Cyanea lamarckii **May**	7	-21.4 (0.2)	8.6 (0.6)	3.9 (0.1)
Cyanea lamarckii **June**	21	-19.5 (0.7)	11.5 (1.5)	3.7 (0.4)
Cyanea lamarckii **July**	5	-19.4 (0.8)	12.1 (1.3)	3.7 (0.3)
Cyanea lamarckii **Aug**	3	-19.2 (0.8)	11.5 (0.8)	3.7 (0.2)
Overall mean *C. lamarckii*	36	-19.8 (1.0)	11.0 (1.8)	3.7 (0.3)
Cyanea capillata **May**	2	-21.4 (0.1)	7.7 (0.1)	3.8 (0.1)
Cyanea capillata **June**	13	-19.5 (1.2)	11.0 (2.1)	3.6 (0.4)
Cyanea capillata **July**	14	-19.4 (1.1)	12.8 (1.3)	3.6 (0.2)
Cyanea capillata **Aug**	16	-18.7 (1.6)	13.3 (1.1)	3.5 (0.3)
Overall mean *C. capillata*	43	-19.7 (1.3)	12.4 (1.8)	3.6 (0.1)

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Table 2(on next page)

Summary statistics for least squares regressions

Table 2: Summary statistics for least squares regressions examining relationships between individual jellyfish size and bell stable isotope ratios (mass, length and δ^{15}N data $\log_{10}$ transformed, δ^{13}C data $\log_{10}+40$ transformed). NB: in all cases slopes were significantly different from 1.

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Species	Isotope	Comparison	Intercept (± SE)	Slope (± SE)	R^2	F	P
A. aurita	$\delta^{13}C$ (-21.1 to -17.2 ‰)	Bell diameter (6 to 36 cm)	1.224 (0.019)	0.079 (0.015)	0.39	$F_{1,41} = 26.3$	< 0.001
A. aurita	$\delta^{15}N$ (6.7 to 14.8 ‰)	Bell diameter (6 to 36 cm)	0.609 (0.056)	0.305 (0.045)	0.53	$F_{1,41} = 46.2$	< 0.001
A. aurita	$\delta^{13}C$ (-21.1 to −17.2 ‰)	Wet mass (12 − 1 702 g)	1.256 (0.013)	0.029 (0.006)	0.40	$F_{1,41} = 26.9$	< 0.001
A. aurita	$\delta^{15}N$ (6.7 to 14.8 ‰)	Wet mass (12 − 1 702 g)	0.730 (0.038)	0.111 (0.016)	0.54	$F_{1,41} = 48.8$	< 0.001
C. lamarckii	$\delta^{13}C$ (-21.6 to -18.5 ‰)	Bell diameter (4 to 20 cm)	1.287 (0.019))	0.018 (0.019)	0.02	$F_{1,35} = 0.85$	= 0.363
C. lamarckii	$\delta^{15}N$ (7.7 to 15.8 ‰)	Bell diameter (4 to 20 cm)	0.939 (0.067)	0.103 (0.066)	0.06	$F_{1,35} = 2.4$	= 0.131
C. lamarckii	$\delta^{13}C$ (-21.6 to -18.5 ‰)	Wet mass (3 to 493 g)	1.293 (0.013)	0.006 (0.007)	0.02	$F_{1,35} = 0.71$	= 0.405
C. lamarckii	$\delta^{15}N$ (7.7 to 15.8 ‰)	Wet mass (3 to 493 g)	0.985 (0.047)	0.030 (0.025)	0.04	$F_{1,35} = 1.50$	= 0.229
C. capillata	$\delta^{13}C$ (-21.8 to -17.2 ‰)	Bell diameter (6 to 85 cm)	1.233 (0.020)	0.062 (0.014)	0.32	$F_{1,41} = 19.1$	< 0.001
C. capillata	$\delta^{15}N$ (7.6 to 16.1 ‰)	Bell diameter (6 to 85 cm)	0.876 (0.046)	0.157 (0.034)	0.34	$F_{1,41} = 22.0$	< 0.001
C. capillata	$\delta^{13}C$ (-21.8 to -17.2 ‰)	Wet mass (19 to 23 680 g)	1.259 (0.015)	0.020 (0.005)	0.28	$F_{1,41} = 16.1$	< 0.001
C. capillata	$\delta^{15}N$ (7.6 to 16.1 ‰)	Wet mass (19 to 23 680 g)	0.931 (0.035)	0.055 (0.012)	0.35	$F_{1,41} = 22.1$	< 0.001

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Table 3(on next page)

Bayesian comparisons of isotopic niche width (SEAB) between different jellyfish species and survey months

Table 3: Table showing results of Bayesian comparisons of isotopic niche width (SEAB) between different jellyfish species and survey months. Probabilities (based on 100,000 draws) that isotopic niche area in Group A is larger than the comparative value in Group B (A>B) are shown. Species codes: *A. a = A. aurita*; *C. l = C. lamarckii*; *C. c = C. capillata*). Groups marked with * reflect samples sizes < 10.
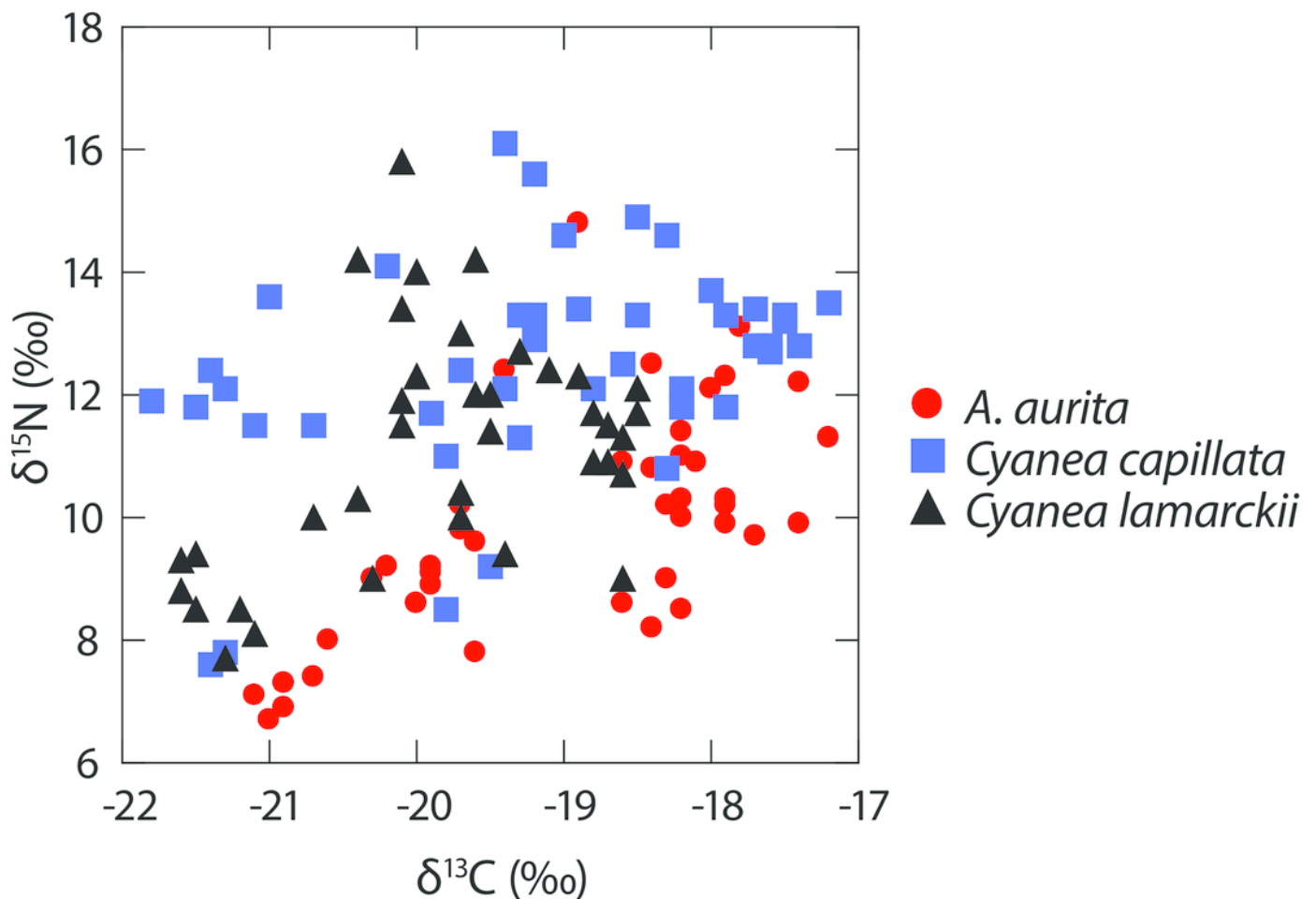
Group		A. a May	A.a June	A. a July*	C. l May*	C. l June	C. l July*	C. c June	C. c July	C. c August
							Group A			
	A. a May	—	0.951	0.980	0.388	0.996	0.969	0.998	0.999	0.999
	A. a June		—	0.756	0.062	0.855	0.728	0.927	0.938	0.969
	A. a July*			—	0.029	0.540	0.496	0.697	0.703	0.775
	C. l May*				—	0.988	0.964	0.993	0.994	0.997
Group B	C. l June					—	0.460	0.713	0.722	0.821
	C. l July*						—	0.683	0.688	0.754
	C. c June							—	0.497	0.596
	C. c July								—	0.609
	C. c August									—

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Isotopic variation in 3 species of co-occuring jellyfish
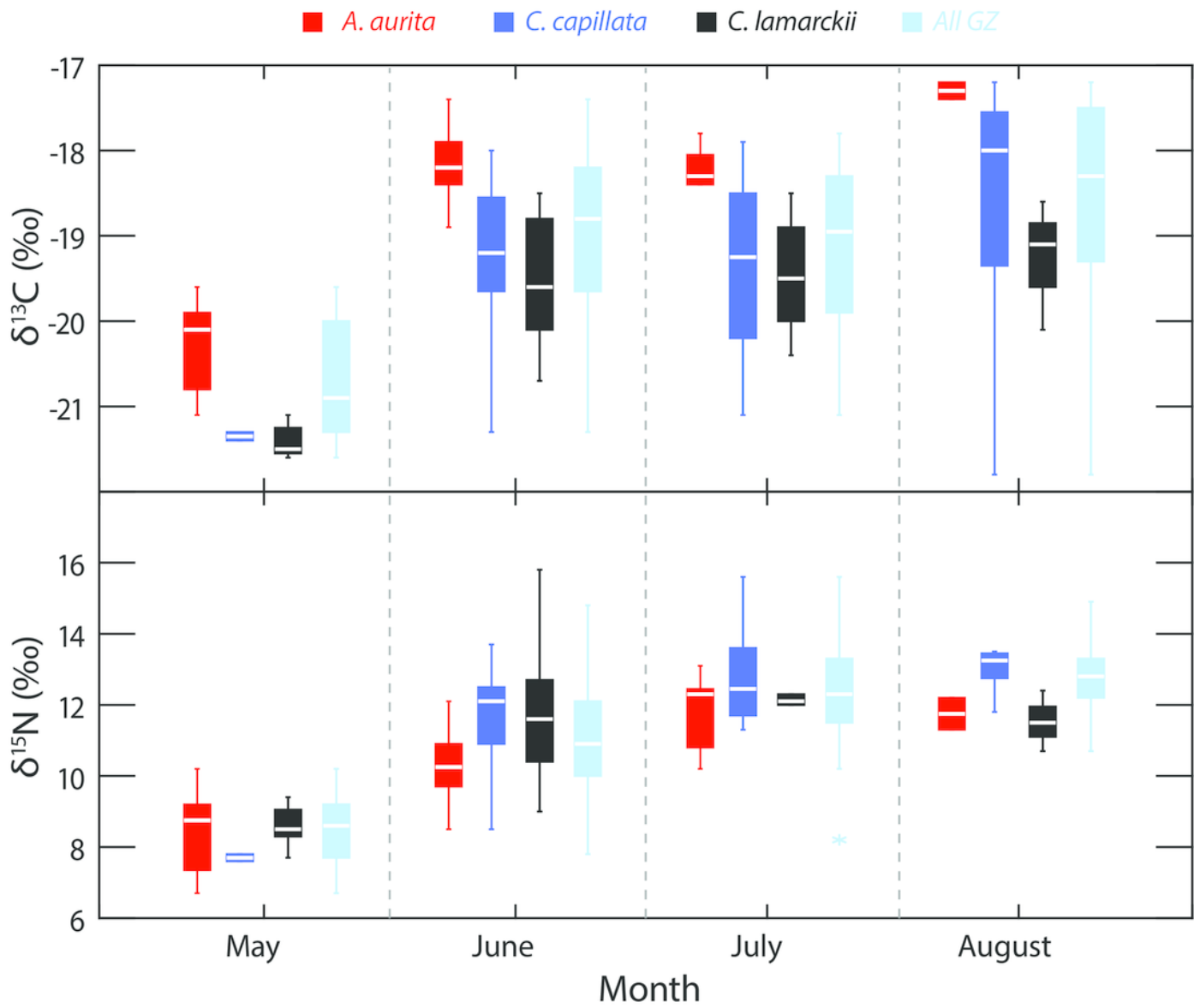
Figure 1: Variation in δ^{13}C and δ^{15}N shown in three species of jellyfish over the whole study period. (See Table 1 for summary statistics).

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Temporal variation in jellyfish $\delta^{13}C$ and $\delta^{15}N$
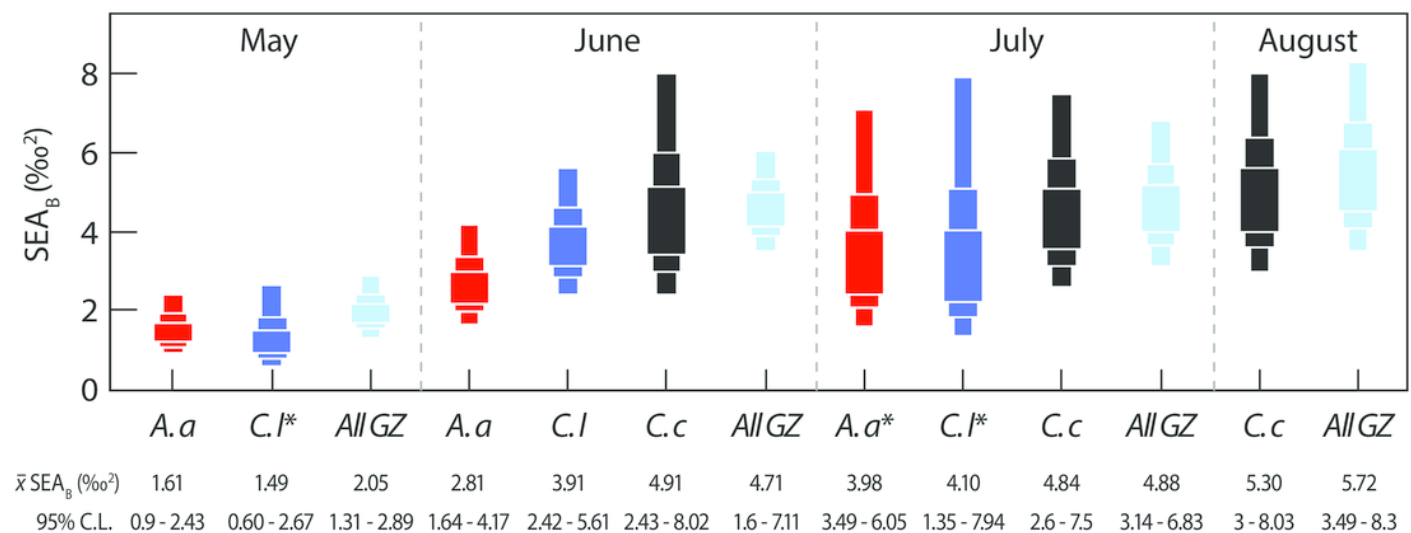
Figure 2: Box-whisker plots showing variation in $\delta^{13}C$ (upper panel) and $\delta^{15}N$ (lower panel) in the three jellyfish species, and within the dominant gelatinous zooplankton community (GZ; all three species combined) over the study period. See Table 1 for sample sizes and other summary statistics. NB: Baseline $\delta^{15}N$ values remained constant over this period, indicating that the increase in $\delta^{15}N$ values reflected a shift in trophic position rather than seasonal shifts at the base of the food web. Boxes show inter-quartile range, and the bold horizontal bar indicates the median value. Whiskers reflect values 1.5 x the interquartile range.

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Variation in isotopic niche width (SEA$_B$) between species

Figure 3: Variation in isotopic niche width (SEA$_B$) between species (*A. a = A. aurita*; *C. l = C. lamarckii*; *C. c = C. capillata*)) and within the dominant gelatinous zooplankton community (GZ; all three species combined) sampled over the survey period. Boxes represent the 50, 75 and 95 % Bayesian credibility intervals estimated from 100,000 draws. Samples marked with *included less than 10 individuals (see Parnell et al. 2010). See Table 3 for statistical comparisons.

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Figure showing isotopic variation with size

Figure 4: Variation in bell $\delta^{13}C$ (A & B) and $\delta^{15}N$ (C & D) with bell diameter (A & C) and wet mass (B & D). Note use of logarithmic scale on x-axes.