NON-LETHAL DORSAL FIN SAMPLING FOR STABLE ISOTOPE ANALYSIS IN SEAHORSES

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Abstract

Sampling collection for stable isotope analysis has traditionally involved the sacrifice of the animal. Seahorses (*Hippocampus* spp.) are listed as threatened by the Convention on International Trade in Endangered Species (http://www.cites.org) and consequently lethal sampling is undesirable. We evaluated the adequacy of dorsal fin tissue of adult seahorses *Hippocampus guttulatus* for stable isotope analysis as an alternative to lethal tissue sampling. Three seahorse tissues (dorsal fin, muscle and liver) were analysed for comparisons of $\delta^{15}N$ and $\delta^{13}C$ values. Similarities found between $\delta^{15}N$ and $\delta^{13}C$ values in dorsal fin and muscle tissue of *H. guttulatus* suggests that both tissues are adequate for stable isotope analysis to understand feeding ecology of seahorses. However, considering the threatened status of the species, dorsal fin tissue would be recommended in adult seahorses as a non-lethal sampling. The effect of lipid extraction on carbon and nitrogen stable isotope values was also evaluated in each seahorse tissues. Significant effects of lipids extraction did only occur for $\delta^{13}C$ values in muscle and liver. It was found that lipid removal was not necessary to perform SIA in

dorsal fin tissues. Due to the limited availability of fin tissue obtained from fin-clipping in seahorses, the relationship between the mass/surface of dorsal fin clip and stable isotope values was analysed. $\delta^{15}N$ and $\delta^{13}C$ values in fin samples were found to be independent of the size of fin analysed. According to our study, the use of fin-clipping sampling, with a minimum surface analysed of 12.74 mm^2, was found to be an adequate method for SIA in seahorses.

Key words: stable isotopes; diet; non-lethal sampling; seahorses; *Hippocampus guttulatus*.

Introduction

The study of the trophic ecology in fishes by means of stable isotope analysis (SIA) has been extensively used over the last two decades (Hobson and Welch 1992; Cabana and Rasmussen 1996; Jennings et al. 1997; Frediksen 2003; Vizzini and Mazzola 2009), since isotopic composition of a consumer's tissue can be correlated with that in the diet (DeNiro and Epstein 1978; 1981). Nitrogen and carbon stable isotopes are commonly used to study food webs, as nitrogen stable isotope ratios ($^{15}N/^{14}N$) are an indicator of a consumer's trophic position, while carbon stable isotope ratios ($^{13}C/^{12}C$) indicate potential sources of food consumed (Peterson and Fry 1987; Hobson and Welch 1992).

Generally, the sampling of fish tissues (muscle, liver, heart, etc.) for stable isotope analysis (SIA) requires the sacrifice of the animal (Hobson and Welch 1992;

Cabana and Rasmussen 1996; Jennings et al. 1997; Frediksen 2003; Vizzini and Mazzola 2009). The use of a non-lethal sampling to measure stable isotope values in studies with threatened or endangered species, such as seahorses (included in the IUCN Red List Category and Criteria) (IUCN, 2011), would be more than suitable as an alternative to lethal sampling procedures. Furthermore, it would allow the study of food webs in seahorses without affecting wild populations, which has a high conservation value.

Fin-clipping is a non-lethal sampling method which requires minimal equipment, handling time, and training. It has been widely used in fisheries and research for identification, contaminant analysis and genetics analysis purposes (Gunnes and Refstie 1980; Wilson and Donaldson 1998; Heltsley et al. 2005). In recent years, fin tissue sampling has become a useful non-lethal tool used in SIA of fish (Jardine et al. 2005; Kelly et al. 2006; Sanderson et al. 2009; Jardine et al. 2011) instead of lethal sampled tissues. In seahorses, fin-clipping has also been used to obtain tissue for genetic analysis and has been shown to have no significant effects on survival (Kvarnemo et al. 2000; Lourie 2003; Pardo et al. 2007). This sampling procedure can also be advisable for SIA in seahorses due to seahorse's capacity for fin regeneration, in around one to two months (Planas et al. 2008). Therefore, fin-clipping could be an adequate non-lethal sampling method for stable isotope analysis in seahorses.

In seahorses, the limited availability of tissue obtained from fin-clipping makes necessary a previous assessment of sample size to evaluate its specific use in SIA. In addition, comparisons of stable isotope values of different tissues should be performed to assess differences among seahorse tissues because isotope values can show variability among tissues due to isotopic fractionation occurring in different tissues (DeNiro and Epstein 1978; 1981; Pinnegar and Polunin 1999). Previous studies

76 performed in other fish species (e.g. slimy sculpin, atlantic salmon, brook trout) have
77 demonstrated that stable isotope values of fin and muscle tissues are correlated (Jardine
78 et al. 2005; Kelly et al. 2006; Jardine et al. 2011), supporting the use of fin tissue as a
79 convenient sample for food web studies using stable isotope analysis.

80 The aim of the study was to establish a sampling and analysis procedure to
81 ensure accurate and reproducible analysis of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in tissues
82 of adult seahorse *Hippocampus guttulatus*. Considering the conservation concern of
83 seahorses, the main objective of this study was to determine the suitability of a non-
84 lethal sampling procedure (fin tissue) and compare it to the use of lethal tissue sampling
85 (liver or muscle). Firstly, three types of tissue (muscle, liver and fin) were compared for
86 SIA in order to assess the adequacy of fin tissue in further studies. Secondly, the effects
87 of lipid extraction on the carbon and nitrogen stable isotope values in seahorse tissues
88 (muscle, liver and fin) was evaluated, as it is known that the lipid content in tissues can
89 potentially affect carbon stable isotope values (DeNiro and Epstein 1978; Pinnegar and
90 Polunin 1999). Finally, the dependency of the isotope values in dorsal fin samples on
91 the sample size of the fin was evaluated. As an application in the field, we provide
92 results of stable isotopes in wild seahorses of the Galician coast (NW Spain).
93
94 Material and methods
95
96 All tissue samples used in this study were taken from six freshly deceased
97 seahorses *Hippocampus guttulatus* of the broodstock maintained at the Instituto de
98 Investigaciones Marinas (CSIC) (Vigo, NW Spain). The analysed seahorses did not
99 show evidence of disease nor external or internal lesion. The diet of the captive
100 seahorses consisted of adult enriched *Artemia* (EG, Inve, Spain), with a $\delta^{13}C$ value of -

101 19.31‰ and a $\delta^{15}N$ value of 3.79‰, offered *ad libitum* twice daily, over a two years
102 period.

103 Seahorses were frozen immediately after dead and stored at -20ºC until
104 processing. Three types of tissue were analysed: muscle, liver and whole dorsal fin (n=6
105 per each tissue type). Muscle and liver tissue are the most common tissues used to
106 obtain long term or short term, respectively, dietary information by stable isotopes
107 analysis (SIA). Muscle has a low-medium lipid content, while liver has high lipid
108 content. Dorsal fin samples were selected to assess their adequacy for trophic ecology
109 studies of seahorses. Muscle and liver samples require the sacrifice of the fish, whereas
110 fin-clipping is a non-lethal sampling procedure. The tissues were removed from each
111 seahorse for lipid extraction assessment and tissues comparison. Each sample was
112 freeze-dried and split into two similar subsamples. One of the subsamples was
113 submitted to lipid extraction following a modification of the procedure described by
114 Bligh and Dyer (1959) (Fernández-Reiriz et al. 1989). Lipids were first extracted with
115 chloroform:methanol (1:2) and after centrifugation (3246 x g), the lipids of the resulting
116 sediment were extracted again with chloroform:methanol (2:1). Finally, both
117 supernatants were washed with chloroform:methanol:water (8:4:3) (Folch et al. 1957).
118 Total lipids content was quantified gravimetrically according to Herbes and Allen
119 (1983). Both subsamples, with or without lipids, were submitted to SIA.

120 Whole dorsal fins (n=6) were also taken and cut off into three sections differing
121 in size (from smaller to larger: DF1, DF2 and DF3) (Fig. 1). The surface of each section
122 was measured from digital photographs using image processing software (NIS
123 Elements, Nikon). Samples were rinsed with distilled water, frozen, freeze-dried and
124 stored at -20ºC until further analysis.

125 In tissue processing, muscle and liver samples were ground to a powder,
126 whereas dorsal fin samples were cut off with scissors into small pieces, except small
127 portions of dorsal fin (DF1) which were used intact. Tissue samples were taken and
128 weighted into tin capsules (1 mg of muscle and liver; 0.2 mg – 1 mg of dorsal fin). The
129 samples were analysed for stable carbon and nitrogen isotopes using an elemental
130 analyser FlashEA 1112 connected to a Thermo-Finnigan MAT 253 mass spectrometer
131 (CACTI, Universidade de Vigo), with an analytic precision of ±0.04‰ for C and
132 ±0.10‰ for N (n=10). Stable isotope values were expressed in conventional delta
133 notation (δ) as parts per thousand (‰) according to the following equation: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X is ^{13}C or ^{15}N and R is the corresponding
134
135 ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. Peedee Belemnite (PDB) and atmospheric
136 nitrogen (AIR) were used as reference material for carbon and nitrogen, respectively.
137 Standards of acetanilide, sulphate ammonia, urea, sucrose and polyethylene were used
138 for system calibration and weighted accordingly to samples weight variability.
139 Stable isotope values were checked for normality using the Shapiro-Wilk test.
140 Paired t-tests were applied to assess differences in $\delta^{13}C$ and $\delta^{15}N$ values between lipid
141 extracted samples and non-lipid extracted samples of muscle, liver and dorsal fin
142 tissues. A repeated measures ANOVA test was applied to check for differences among
143 tissues. When significant differences were found among tissues ($p < 0.05$), a Bonferroni
144 post-hoc test was applied. Relationships between weight and isotope values of dorsal fin
145 were analysed using linear regressions. All the analyses were performed using the
146 statistical package SPSS v.15.0.
147
148 Results
149

150 *Tissue comparisons*

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152 Tissue comparisons were made using $\delta^{15}N$ values of non-lipid extracted tissues
153 of dorsal fin, liver and muscle, $\delta^{13}C$ values of non-lipid extracted dorsal fin tissue and
154 $\delta^{13}C$ values of lipid extracted samples of liver and muscle. $\delta^{15}N$ values in muscle (11.35
155 ± 0.53) were slightly higher but not significantly different (ANOVA, $F_{2,4} = 0.62$, $p =$
156 0.580) than $\delta^{15}N$ values in dorsal fin and liver (11.05 ± 0.62, 10.67 ± 1.39, respectively).
157 For $\delta^{13}C$, significant differences were found among tissues (ANOVA, $F_{2,4} = 63.81$, $p <$
158 0.05) (-19.27 ± 0.60 in liver, -17.04 ± 1.07 in dorsal fin and -17.59 ± 1.61 in muscle)
159 (Table 1), although differences were only significant between dorsal fin and liver tissue
160 (Bonferroni post-hoc test, $p < 0.05$).
161 The relationship among tissues for both $\delta^{15}N$ and $\delta^{13}C$ signatures are provided in Fig. 2.

162

163 *Lipid extraction*

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165 The total lipids content (%) and $\delta^{13}C$ and $\delta^{15}N$ values (mean ± sd, ‰) in lipid
166 extracted and non-lipid extracted tissues are summarized in Table 2. Lipid extraction
167 was found to cause no difference in $\delta^{15}N$ and $\delta^{13}C$ values of the dorsal fin; neither did it
168 significantly affect $\delta^{15}N$ values in muscle and liver. However, $\delta^{13}C$ values in both
169 muscle and liver were significantly affected by lipid extraction (Student paired t-test ,
170 $p<0.05$), with an increase after lipid extraction of 0.55 ± 1.61 ‰ for muscle and 2.56 ±
171 0.72 ‰ for liver.

172

173 *Dorsal fin size*

174

175 The mean size of the fin clips sampled were 19.99 ± 9.10 mm^2 in the small
176 section DF1, 64.63 ± 16.15 mm^2 in the intermediate section DF2 and 94.50 ± 20.73
177 mm^2 in the big section DF3. The minimum and maximum fin size analysed were 12.74
178 mm^2 (DF1) and 119.29 mm^2 (DF3), respectively. These portions corresponded to 0.21
179 and 2.15 mg dry weight, respectively. The isotope values were found to be independent
180 of the size of fin analysed (Linear regression, $F_{1,17} = 2.22$, $p = 0.15$, $F_{1,17} = 0.009$, $p =$
181 0.92, for N and C respectively) (Fig. 3). Average values of $\delta^{15}N$ for portions DF1, DF2
182 and DF3 were 11.05 ± 0.62, 10.64 ± 0.12 and 11.63 ± 0.42, respectively, whereas mean
183 values of $\delta^{13}C$ were -17.04 ± 1.07, -16.75 ± 1.07 and -16.96 ± 1.27.
184 For comparative purposes with the values of stable isotopes in the three tissues
185 analysed in the present study from captive seahorses, the values of $\delta^{13}C$ and $\delta^{15}N$ in fin
186 samples of wild seahorses *H. guttulatus* captured at four different sites in the Galician
187 coast (NW Spain) are shown in Fig. 4.
188
189 Discussion
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191 *Tissue comparison*
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193 Despite the slightly higher $\delta^{15}N$ values found in muscle, the values encountered
194 in the three tissues analysed (muscle, liver and dorsal fin) were not significantly
195 different. McCarthy and Waldron (2000) also reported equivalent values in fin and
196 muscle tissue for $\delta^{15}N$ in brown trout. Similar results were also reported by Kelly et al.
197 (2006) in slimy sculpin, although a correction factor was applied by these authors. On
198 the contrary, a significant enrichment in $\delta^{15}N$ was pointed out in muscle relative to fin
199 tissue in salmon (Jardine et al. 2005; Sanderson et al. 2009). Pinnegar and Polunin

200 (1999) suggested that differences in $\delta^{15}N$ amongst different tissues could be due to their
201 composition in amino acids. The similarity of $\delta^{15}N$ values in the three tissues analysed
202 in this study suggests that all three tissues are suitable for SIA, although dorsal fin
203 would be recommended in alive adult seahorses as a non-lethal method.

204 Lipid rich tissues, such as muscle and especially liver, have lower $\delta^{13}C$ values
205 than other tissues, because lipids tend to be more $\delta^{13}C$ depleted (DeNiro and Epstein
206 1978; Pinnegar and Polunin 1999). Unexpectedly, lipid extraction did not reduce
207 differences in $\delta^{13}C$ values between dorsal fin and liver. As for $\delta^{15}N$, differences among
208 tissues in $\delta^{13}C$ values have been attributed to the amino acid composition in tissues
209 (DeNiro and Epstein 1978). The $\delta^{13}C$ values of seahorse dorsal fin tissue, however,
210 were found to be similar to those in muscle, similarly to previous studies in brown trout
211 (McCarthy and Waldron 2000), Atlantic salmon (Jardine et al. 2005) and tropical fishes
212 (Jardine et al. 2011). Muscle tissue has a slow turnover rate that provides more
213 information over time about the diet when compared to tissues with fast isotopic
214 turnover rate, such as liver (Hobson and Welch 1992). The similarity between $\delta^{15}N$ and
215 $\delta^{13}C$ values of *H. guttulatus* dorsal fin and muscle tissue suggests that both tissues are
216 adequate for SIA to provide dietary information in a relatively long term. In food web
217 studies, the analysis of $\delta^{15}N$ and $\delta^{13}C$ in dorsal fin tissue would constitute a simple and
218 non-lethal sampling procedure providing long-term information on the feeding habits in
219 seahorses.

220

221 *Lipid extraction*

222

223 Compared to other biochemical components, lipids are depleted in $\delta^{13}C$ due to
224 lipid synthesis (DeNiro and Epstein 1977). Hence, the variability of lipid content in

225 different tissues significantly influences $\delta^{13}C$ values in the tissue (DeNiro and Epstein
226 1978; Pinnegar and Polunin 1999). For this reason, tissues submitted to SIA frequently
227 undergo lipid extraction increasing the reliability of the results. Although the effects of
228 lipid extraction in fish tissues have been reported by several authors (Pinnegar and
229 Polunin 1999; Sotiropoulos et al. 2004; Sweeting et al. 2006; Logan et al. 2008), the
230 results achieved are contradictory. No previous studies had been carried out in seahorses
231 and we considered necessary to determine the effects of lipid removal on the
232 quantification of stable isotope in seahorse tissues.

233 According to the results obtained from liver and muscle analysis, the differences
234 found in $\delta^{13}C$ values between lipid extracted and non-lipid extracted tissues agree with
235 previous studies in fish (Pinnegar and Polunin 1999; Sotiropoulos et al. 2004; Sweeting
236 et al. 2006; Logan et al. 2008). Those differences can be explained by the lipid content
237 in muscle (7.1% dry weight) and especially in liver (58.5% dry weight). Therefore, lipid
238 removal seems to be necessary in the analysis of $\delta^{13}C$ in both muscle and liver of
239 seahorses. Conversely, lipid extraction did not affect $\delta^{15}N$ values in either muscle or
240 liver. Similar results were attained by Logan et al. (2008) in salmon, perch and herring.
241 Some studies have reported significant differences in $\delta^{15}N$ values associated to lipid
242 extraction in muscle and liver tissues of fish (Sotiropoulos et al. 2004; Sweeting et al.
243 2006). These findings were related to the solvent effect. Therefore, in some cases the
244 analysis of stable isotopes would require a preliminary lipid extraction in the samples
245 depending on the type of isotope considered, C or N.

246 Regarding dorsal fin, this tissue is composed by a mixture of bone, muscle, and
247 cartilage, containing 2.6% dry weight of lipids. As expected, due to this very low lipid
248 content, lipid removal had no effect on stable isotope values. Consequently, lipid
249 removal in dorsal fin tissue of seahorses would not be necessary to perform SIA. Our

250 results agree with Post et al (2007), who reported that for aquatic animals it is not
251 necessary to account for lipids in samples when lipid content is consistently low (<5%
252 lipids; C:N < 3.5), which is the case of fin samples (2.6% lipids; C:N=3.3).
253
254 *Dorsal fin size*
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256 The minimum amount of C and N required for SIA with the analytical
257 equipment used in the present study was 20 μg and 50 μg, respectively, This
258 requirement was fully satisfied this the smaller section DF1, whose mean content in C
259 and N was 69.98 and 230.10 μg, respectively. Consequently, small sections of dorsal fin
260 with 19.99 ± 9.10 mm^2 surface or 0.21 mg dry weight were perfectly adequate for $\delta^{15}N$
261 and $\delta^{13}C$ analysis. The surface of this section sample is equivalent to 8.66% of total
262 dorsal fin surface.
263 According to our results, fin-clipping in seahorses has important advantages over
264 the use of other tissues: i) It is a non-lethal sampling procedure, ii) it does not require
265 lipid removal in the tissue, iii) the fin is regenerated in one-two months (Planas et al.
266 2008), allowing multiple fin clips on the same seahorse over time, and iv) it has no
267 effect on growth, survival or ability to swim (unpub. data).
268 Our study was performed in adult seahorses, measuring > 15 cm in total length
269 and the results achieved here cannot be extrapolated to juveniles or newborns, where the
270 full body must be analysed. Further studies would be necessary to assess the application
271 of fin-clipping to SIA according to the age of seahorses.
272 We consider that fin-clipping is an alternative to muscle tissue for SIA in *H.*
273 *guttulatus*, a species with conservation concern and very low population densities. Due
274 to imperative legal limitations (the capture of seahorses was not allowed for sacrifice) in

275 the availability of seahorses, the number of samples available for SIA in this study was
276 very low and restricted to naturally dead animals. Sanderson et al. (2009) pointed out
277 that fin-clipping use provide a useful tracer for ecologists (e.g. to determine dietary
278 sources) and have been found in wild seahorses at the Galician Coast with a relatively
279 low intra-site variability of the isotopic composition. A high number of samples would
280 be necessary to assess a more precise quantification in all aspects of the analysis
281 performed. In spite of this, we consider that the analysis of fin resulted in values
282 equivalent to those of muscle tissue. Average $\delta^{15}N$ (range: 9.94 to 11.71 ‰ in fin and
283 10.74 to 12.08 ‰ in non-lipid extracted muscle) and $\delta^{13}C$ (range: - 18.81 to -15.99 ‰
284 in fin and -19.66 to -17.59 ‰ in lipid extracted muscle) values in fin differed from
285 muscle by 0.30 and 0.55 ‰, respectively. This variation corresponds to 2.7% for $\delta^{15}N$
286 and 3.25% for $\delta^{13}C$, which is much lower than the variation encountered when
287 comparing adult seahorses from the wild with adult seahorses from the laboratory (Fig.
288 4). Sanderson et al (2009) demonstrated that analyzing fins for $\delta^{15}N$ and $\delta^{13}C$ in
289 *Oncorhynchus tshawytscha* and O. *mykiss* would produce results equivalent to those
290 using muscle tissue and that fin $\delta^{15}N$ and $\delta^{13}C$ mimic those of muscle tissue in both time
291 and space. These authors also pointed out that if there is no specific need to quantify
292 isotopes using muscle tissue, muscle and fin tissues are equally powerful, and suggested
293 that new projects can simply collect fin tissue throughout the project duration.
294
295 Conclusions
296
297 Our results provide a framework for using dorsal fin tissue in the measurement
298 of $\delta^{15}N$ and $\delta^{13}C$ in seahorses. We propose fin-clipping as a standard non-lethal

299 sampling method in future stable isotopes studies (SIA) with seahorses, avoiding the use
300 of lethal techniques.
301
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311
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LIST OF FIGURES:

FIGURE 1 Sections with different sizes (from smaller to larger: DF1, DF2 and DF3) of dorsal fin tissue of seahorse *Hippocampus guttulatus*.

FIGURE 2 Relationship between $\delta^{15}N$ and $\delta^{13}C$ values and tissues (fin, liver and muscle) in adult seahorses *Hippocampus guttulatus*. Liver and muscle tissues were lipid extracted for $\delta^{13}C$.

FIGURE 3 Relationship between dry weight (mg) of non-lipid extracted dorsal fin samples (n=6) and $\delta^{15}N$ and $\delta^{13}C$ values of dorsal fin in adult seahorses *Hippocampus guttulatus*. DF1: small portions (n=6); DF2: medium portions (n=6); DF3: large portions (n=6).

FIGURE 4 Boxplot of $\delta^{15}N$ and $\delta^{13}C$ values in dorsal fin samples from wild seahorses *Hippocampus guttulatus* captured at four different sites in the Galician coast (NW Spain) – Site 1 (n=4), Site 2 (n=11), Site 3 (n=4), Site 4 (n=3). Values of $\delta^{15}N$ and $\delta^{13}C$ from three different tissues (dorsal fin, liver and muscle) of captive *Hippocampus guttulatus* seahorses (n=6) are provided for comparative purposes.

417 TABLE CAPTIONS:

418 Table 1 Values of $\delta^{15}N$ and $\delta^{13}C$ and elemental composition in C and N (dry weight %)
419 in dorsal fin, liver and muscle tissues of six adult seahorses *Hippocampus guttulatus*.
420 Mean ± sd, minimum and maximum values are provided for each tissue. See text for
421 further details.

422

423 Table 2 Lipid content (% dry weight) and $\delta^{13}C$ and $\delta^{15}N$ values (mean ± sd) in dorsal
424 fin, liver and muscle tissues of adult seahorses *Hippocampus guttulatus* submitted or not
425 to lipid extraction. Statistic t and level of significance p of the Student paired t-test
426 analysis are provided.

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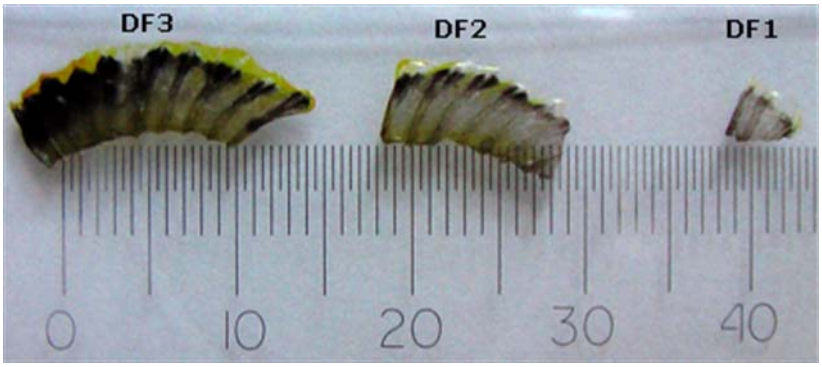
Table 1 Values of $\delta^{15}N$ and $\delta^{13}C$ and elemental composition in C and N (dry weight %) in dorsal fin, liver and muscle tissues of six adult seahorses *Hippocampus guttulatus*. Mean ± sd, minimum and maximum values are provided for each tissue. See text for further details.

	Dorsal Fin		Liver		Muscle		Dorsal Fin		Liver		Muscle	
	$\delta^{15}N$ (‰)	% N	$\delta^{15}N$ (‰)	% N	$\delta^{15}N$ (‰)	% N	$\delta^{13}C$ (‰)	% C	$\delta^{13}C$ (‰)	% C	$\delta^{13}C$ (‰)	% C
	9.94	13.02	11.19	4.87	10.74	14.78	-18.81	41.96	-20.17	49.03	-19.66	40.38
	11.71	11.76	12.21	7.14	11.27	13.75	-17.87	40.64	-19.51	47.54	-19.46	47.24
	11.19	12.21	11.39	3.81	10.8	12.41	-16.41	38.8	-19.51	53.16	-16.92	50.6
	10.81	12.37	8.73	3.58	11.76	13.02	-16.54	40.48	-18.4	46.54	-16.98	46.1
	11.26	15.66	11.34	3.47	12.08	14.55	-16.6	56.32	-19	51.56	-16.89	46.95
	11.38	12.91	9.16	4.41	11.44	12.46	-15.99	42.36	-17.9	45.57	-15.64	45.6
Mean	11.05	12.99	10.67	4.55	11.35	13.50	-17.04	43.43	-19.08	48.90	-17.59	46.15
sd	0.62	1.39	1.39	1.38	0.53	1.03	1.07	6.44	0.83	2.96	1.61	3.32
Minimum	9.94	11.76	8.73	3.47	10.74	12.41	-18.81	38.8	-20.17	45.57	-19.66	40.38
Maximum	11.71	15.66	12.21	7.14	12.08	14.78	-15.99	56.32	-18.4	53.16	-15.64	50.6

Table 2 Lipid content (% dry weight) and $\delta^{13}C$ and $\delta^{15}N$ values (mean ± sd) in dorsal fin, liver and muscle tissues of adult seahorses *Hippocampus guttulatus* submitted or not to lipid extraction. Statistic t and level of significance p of the Student paired t-test analysis are provided.

		$\delta^{13}C$ (‰)				$\delta^{15}N$ (‰)			
Tissue (n)	% lipids	Non-lipid extraction	Lipid extraction	t	p	Non-lipid extraction	Lipid extraction	t	p
Dorsal fin (6)	2.6 ± 2.3	-18.24 ± 0.63	-17.98 ± 0.53	-1.50	0.207	10.80 ± 0.36	10.90 ± 0.44	-0.48	0.658
Muscle (6)	7.1 ± 4.0	-18.14 ± 1.62	-17.59 ± 1.60	-6.59	0.001	11.35 ± 0.53	11.96 ± 0.89	-2.05	0.096
Liver (6)	58.5 ± 16.2	-21.64 ± 0.61	-19.08 ± 0.83	-19.07	<0.001	10.67 ± 1.39	10.67 ± 0.50	-0.003	0.998


DF3
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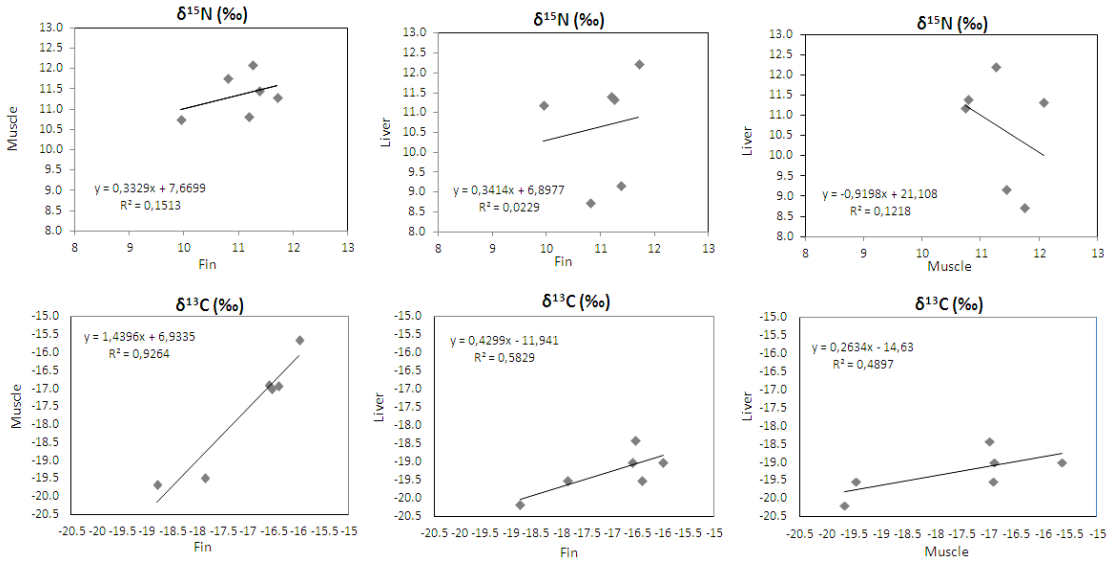
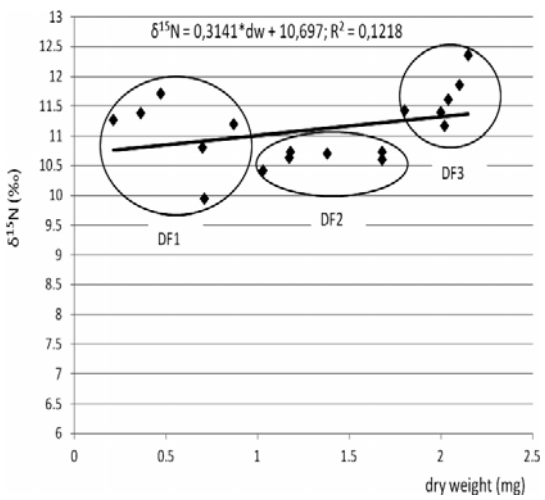
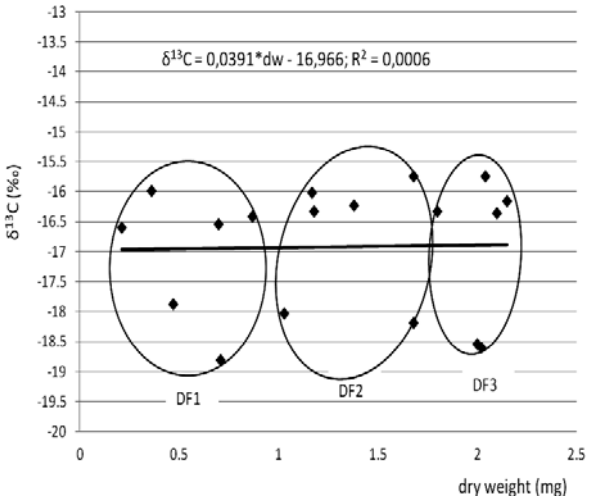

δ15N (‰)
Muscle
Fin
y = 0,3329x + 7,6699
R² = 0,1513
δ15N (‰)
Liver
Fin
y = 0,3414x + 6,8977
R² = 0,0229
δ15N (‰)
Liver
Muscle
y = -0,9198x + 21,108
R² = 0,1218
δ13C (‰)
Muscle
Fin
y = 1,4396x + 6,9335
R² = 0,9264
δ13C (‰)
Liver
Fin
y = 0,4299x - 11,941
R² = 0,5829
δ13C (‰)
Liver
Muscle
y = 0,2634x - 14,63
R² = 0,4897





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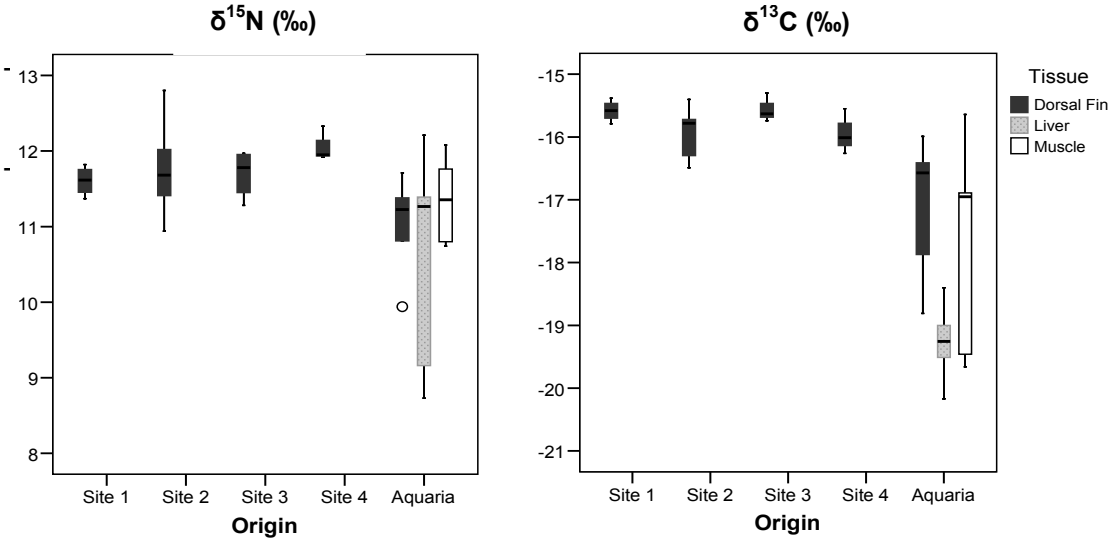

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