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Underwater tagging of the Atlantic Bluefin Tuna in the trap Fishery of Sardinia (W Mediterranean) --Manuscript Draft--

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Abstract:	<p>Tagging bluefin tuna (BFT) has become an essential tool for fishery science that has improved the identification of growth parameters and age validation, population abundance estimates, movement and migration patterns and spatial and temporal population dynamics. Although innovative technologies and methodologies have been introduced, some unresolved issues regarding the possible alteration of fish behaviour and survival during post-tagging as consequence of capture and handling. Such issues have raised the question of whether underwater tagging might be less invasive and preferable to tagging fish on board. In the present study a framework to manage traditional trap gear "Tonnara" for underwater tagging and release purposes was developed for conventional tagging in the Sardinian traps. The general objective of the current study was to ameliorate the operational framework to determine best practices for the underwater tagging of BFT using pneumatic spearguns. Our specific objectives were: (1) to identify the proper size of pneumatic speargun and its operating pressure, (2) to identify the proper shooting distance for the aforementioned equipment, (3) to develop a tool for the indirect estimate of tuna size during tuna tagging operations and 4) to report the results of the tagging activities carried out with conventional tags in Sardinian traps during the 2014 season. The results of the penetration test showed that the shooting distance should be 1-3 m to be successful using a pneumatic speargun at 20 bars of pressure. The indirect length estimation of BFT size was more accurate when the lasers were exactly perpendicular to the animal. However, this method always underestimates the size of the fish, with an average relative error of about -30 cm. During tagging activities in the Sardinian trap in 2014, a total of 63 fish were tagged in 3.5 hours, and only one fish died directly from tagging injuries. The trap represents an optimal system for tagging large numbers of BFT in confined waters when the main goal is to release the fish in the best possible condition. The fish can be confined for several hours in the death chamber, allowing determination of the survival of tagged fish and tag retention.</p>

Underwater tagging of the Atlantic Bluefin Tuna in the trap Fishery of Sardinia (W Mediterranean)

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This work was carried out in accordance with The protocol and procedures are full in accordance with the European [Directive 2010/63/EU](#) for animal experiments.

Highlights

Tagging from the Mediterranean traps, the ancient fishery for bluefin tuna.

An operational framework to handle the trap and enable underwater tagging with speargun is described.

63 bluefin tunas were tagged underwater during their spawning migration in one day.

Length estimation by laser pointers underestimate the actual size of bluefin tunas.

Bluefin tuna confined for several hours allow to determine the survival of fish after the tagging.

1 **Underwater tagging of the Atlantic Bluefin Tuna in the trap Fishery of Sardinia**
2 **(W Mediterranean)**

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24 **Abstract**

25

26 Tagging bluefin tuna (BFT) has become an essential tool for fishery science that has improved the
27 identification of growth parameters and age validation, population abundance estimates, movement
28 and migration patterns and spatial and temporal population dynamics. Although innovative
29 technologies and methodologies have been introduced, some unresolved issues remained regarding
30 the possible alteration of fish behaviour and survival during post-tagging as consequence of capture
31 and handling. Such issues have raised the question of whether underwater tagging might be less
32 invasive and preferable to tagging fish on board. In the present study a framework to manage
33 traditional trap gear “*Tonnara*” for underwater tagging and release purposes was developed for
34 conventional tagging in the Sardinian traps. The general objective of the current study was to
35 ameliorate the operational framework to determine best practices for the underwater tagging of BFT
36 using pneumatic spearguns. Our specific objectives were: (1) to identify the proper size of pneumatic
37 speargun and its operating pressure, (2) to identify the proper shooting distance for the
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39 tagging operations and 4) to report the results of the tagging activities carried out with conventional
40 tags in Sardinian traps during the 2014 season. The results of the penetration test showed that the
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43 perpendicular to the animal. However, this method always underestimates the size of the fish, with
44 an average relative error of about –30 cm. During tagging activities in the Sardinian trap in 2014, a
45 total of 63 fish were tagged in 3.5 hours, and only one fish died directly from tagging injuries. The
46 trap represents an optimal system for tagging large numbers of BFT in confined waters when the main
47 goal is to release the fish in the best possible condition. The fish can be confined for several hours in
48 the death chamber, allowing determination of the survival of tagged fish and tag retention.

49

50 *Keywords:* *Thunnus thynnus*; conventional tagging; size estimation; laser metrics; trap fishery

51

52 1. Introduction

53

54 The tagging of Atlantic bluefin tuna (BFT), *Thunnus thynnus* has long been recognised as a valuable
55 means of studying key aspects of its biology, including its life history, migration, movements and
56 population structure (Abascal et al., 2016; Block et al., 2005, 1998; Cermeño et al., 2015; Cort, 1990;
57 Galuardi and Lutcavage, 2012; Sibert et al., 2006; Stokesbury et al., 2007; Teo et al., 2007a; Walli et
58 al., 2009). Tagging programs were conducted in a systematic way in the Eastern Atlantic,
59 Mediterranean Sea and Northwest Atlantic (Block, 1998; Block et al., 2005, 2001; Boustany et al.,
60 2008; Kitagawa et al., 2007; Magnuson et al., 1994; Stokesbury et al., 2004, 2007). In the Eastern
61 Atlantic and Mediterranean Sea, BFT tagging campaigns began in the early 1900s, and a significant
62 increase in numbers of tagged fish was achieved in 1960–80 (Stokesbury et al., 2007; Tičina et al.,
63 2007, 2004; Yamashita and Miyabe, 2001). In the last ten years, the International Commission for
64 the Conservation of Atlantic Tunas (ICCAT), under the Atlantic-Wide Research Programme for
65 Bluefin Tuna (GBYP), has sustained the collection of fisheries-independent data, as well as the
66 improvement of information on stock structure and fish movements (ICCAT, 2013). Since 2010, the
67 GBYP Programme has addressed the necessity of understanding key biological and ecological
68 processes, assessment models and provision of scientific advice on stock status through conventional
69 and electronic tagging. Such activities have been strongly stressed by the scientific community to
70 improve information on the connectivity between Western–Eastern Atlantic stocks and vice versa,
71 and with the Mediterranean Sea (Abascal et al., 2016; Cermeño et al., 2015; Fromentin and
72 Lopuszanski, 2014; Rouyer et al., 2020).

73 The tagging of BFT involves three sequential phases: fish capture, tag insertion and release. Each
74 phase may be achieved with different tools and techniques for handling the fish, a process that may
75 cause physical injuries and stress or alter fish behaviour in tagged fish. Fish removed from their
76 natural environment and from the school may gain injuries from hooking and is undergone to lower
77 levels of oxygen. This can increase their disorientation and vulnerability to predation, thus nullifying
78 the tagging (Hoyle et al., 2015; Skomal and Chase, 2002). One of the main concerns is the post-
79 release phase, because if these impacts occur at that time, they are unrecognizable, particularly when
80 fish are released after exhausting capture (Davenport et al., 2002).

81 In the Western Atlantic (Eastern North America), most of the tagging programs carried out on
82 BFT have used the rod and reel technique, tagging hooked fish on board (Block, 1998; Brill et al.,
83 2002; Lawson et al., 2010; Sibert et al., 2006; Stokesbury et al., 2011; Teo et al., 2007b; Walli et al.,
84 2009). This technique has excellent results in terms of number of BFT tagged owing to the substantial
85 abundance of BFT in this area during winter months (Block et al., 1998). A previous study reported

86 the capture, tagging and release of 20–60 fish per day. However, the achievement of such numbers is
87 unrealistic for the Mediterranean Sea. In fact, because of the spreading behaviour of BFT during the
88 winter, the strike ratio of fishermen using a rod and reel is only 0–5 specimens per day (FIPSAS,
89 2019).

90 A promising technique for both successful tagging and animal welfare is to tag BFT directly
91 underwater, preferably in confined waters where entrapped fish can be kept for scientific purposes.
92 Information on the underwater tagging of BFT in the Eastern Atlantic and Mediterranean are scant
93 and dated. Two studies focused on tagging of farmed tunas to investigate their growth performance
94 in cages (Tičina et al., 2004, 2003), while the pioneering investigation of De Metro et al. (2002)
95 investigated the post-spawning behaviour of BFT tagged with pop-up satellite tags. More recently,
96 underwater tagging has been advanced by purse seine or traditional traps for ICCAT programs
97 (Abascal et al., 2016; Addis et al., 2014; Karakulak et al., 2015; Mariani et al., 2016).

98 Sardinia (Western Mediterranean), the second largest island in the Mediterranean Sea, is
99 geographically located along the reproductive migration pathway of the Atlantic BFT (Addis et al.,
100 2016a). From late April until mid-July, tuna schools migrate along the western coastline in a
101 southward direction, swimming near the bathymetric contour of 40 m and lower, where they have for
102 centuries been intercepted by the local trap fishery *tonnara* (Addis et al., 2012). This fishery is the
103 only remaining commercially active trap fishery in the Mediterranean which, together with those on
104 the Eastern Atlantic coasts of Spain, Morocco and Portugal, provides stationary scientific data about
105 the status of the BFT population (ICCAT, 2012). These fisheries have implemented national and
106 international scientific monitoring programs to study the biology and ecology of BFT, including
107 conventional and electronic tagging activities (Di Natale et al., 2018).

108 The first conventional tagging in the Sardinian trap was carried out during the 2013 fishing season.
109 This campaign resulted in the tagging and release of 250 BFT. Although this tagging program had
110 satisfactory results in terms of number of fishes tagged, some methodological limitations occurred.
111 These included tag applicator retention, speargun ballistics (size and air charge features), shooting
112 distance, fish length estimation and effectiveness of the trap fishery for underwater tagging of BFT
113 (Addis et al., 2014). These preliminary results have encouraged new experimental tagging methods
114 to ameliorate the operational framework for the underwater tagging of BFT in traditional traps, which
115 was the general objective of the current study.

116 The specific objective of the present study was to evaluate the effectiveness of underwater tagging,
117 considering the effect of the following factors on the penetration and retention of the tag: 1) speargun
118 size; 2) shooting distance; 3) tuna size; 4) shape of the tag applicator; and 5) diver skill in terms of

119 good/bad shots. Moreover, the accuracy of indirect length estimation of tuna size by laser pointers
120 was evaluated considering the effect of distance and angles.

121 Finally, we report here the results of tagging activities in a Sardinian trap fishery during the 2014
122 season to evaluate the suitability of traditional traps (*tonnara*) for underwater tagging of BFT.

123

124 **2. Material and Methods**

125

126 *2.1. Study area and trap-gear adjustment for tagging*

127

128 The study was conducted in southwestern Sardinia (Italy; 39°11'N, 08°18'E). Here the environmental
129 features have made the area suitable for BFT occurrence and fisheries since the sixteenth century,
130 when the first *tonnara* trap was documented (Addis et al., 2016a).

131 The trap is classified as a *tonnara di corsa* (arrival trap) because BFT are captured along their pre-
132 spawning migration route with ripening gonads. The fishing gear consisted of five chambers settled
133 at 42 m depth with a 1050 m long tail (Fig. 1). The chamber used for the experimental tagging
134 corresponds to the death chamber (*camera della morte*), which has a moving net floor, the *corpus*.
135 This is the chamber where the *mattanza* generally took place. The *corpus* is a net floor handled by
136 fishermen (pull and cast net) that allows regulation of water volume and depth to maintain caged
137 BFT. This feature permits users to keep tunas caged in a free-swimming state and to check their stress
138 conditions, which can occur before and after tagging activities (Addis et al., 2013a). The trap crew is
139 comprised of 25 fishermen. In mid-April, the trap fishery is fully operational, and the earliest
140 entrapment of BFT occurs in late April. The gear reaches its maximum capture production in mid-
141 May, after which the number of fishes entrapped decreases due to the progressive ending of the BFT
142 reproductive migration (Addis et al., 2013b).

143

144 *2.2. Test of tag penetration by pneumatic spearguns*

145

146 The applicators for conventional tagging designed by ICCAT utilize three different systems: single
147 barb spaghetti (FT), double barb (FIM) and large billfish double barb (BFIM) tag applicators. These
148 applicators have been specifically designed for the insertion of tags by hand, so control of pressure
149 when inserting the applicator into the BFT body is the responsibility of the tagger. Previous tests have
150 shown that FT and FIM applicators are not suitable for speargun tagging because of breakage of the

151 nylon tag with FT applicators and breakage of the thin steel tip with FIM applicators (Addis et al.,
152 2014).

153 For this reason, BFIM applicators were used for the penetration test. The trial was conducted using
154 different pneumatic spearguns (Mares, mod. Cyrano HTM Sport GmbH, Austria) of 85, 97 and 110
155 cm total length with an operating air pressure of 20 bars. All spearguns were equipped with a 7 mm
156 \varnothing shaft modified for a BFIM applicator. Tag penetration into the BFT body was evaluated
157 considering the tag applicator with a stopper (Y) and without a stopper (I). Three shooting distances
158 (1, 3 and 5 m) were tested.

159 The penetration test was carried out on a sample of six dead BFT belonging to two size classes:
160 small (S) (127–150 cm fork length; n = 3 fish) and giant (G) (212–235 cm fork length n = 3).
161 Experiments were carried out with tunas placed on the sea bottom at 5 m depth. The placement point
162 for tagging was at the base of the second dorsal fin of the BFT (Fig. 2), which corresponds to the
163 conventional point for tagging tunas (Cort et al., 2010). Specimens of BFT used for the penetration
164 test were supplied by the trap company and consisted of fish killed by entanglement in the trap nets.

165 The penetration capacity of the BFIM applicator in the tuna body was categorized as follows: Too
166 Deep (TD), Deep (DP), Correct Position (CP), Not Penetrate (NP) and Not Reach the target (NR).
167 The tag was in the TD position when the tag was not visible and was completely embedded into the
168 muscle of the fish. The tag was in the DP position when the tag was partially embedded into the
169 muscle of the fish and the tag code was not visible. The tag was in the CP position when the anchor
170 was completely embedded into the muscle of the fish and the tag code was entirely visible. The tag
171 was considered NP as a result of unsuccessful anchoring on the tuna body. When the shaft did not
172 reach the fish, it was categorized as NR.

173 A pairwise Fisher's exact test ($\alpha = 0.05$) was used to compare the penetration levels among
174 shooting distances (1 m vs. 3 m, 1 m vs. 5 m, 3 m vs. 5 m), speargun length (85 cm vs. 97 cm, 97 cm
175 vs. 110 cm, 85 cm vs. 110 cm), applicators (I vs. Y) and BFT size (S vs. G).

176

177 *2.3. Length validation by laser measurements*

178

179 The laser device used for the length estimation employs the same principle described in other studies
180 (Deakos, 2010; Rohner et al., 2011; Rowe and Dawson, 2008). Those authors used two parallel lasers
181 (horizontally mounted) at a known distance apart on a fixed camera-speargun base. In the present
182 study, we used two green laser pointers for SCUBA diving (Apinex model BALP-LG05-B150,
183 Montreal, Canada; waterproof up to 300 feet; wavelength 532 nm). The laser pointers were assembled
184 using a LexanTM holder with an inter-distance of 9 cm. The holder had calibration screws to adjust

185 and maintain the laser beams at a constant inter-distance. To avoid a parallax error, the lasers' inter-
186 distance was calibrated from the water projecting laser beams on a wall at 1, 3 and 5 m, respectively.
187 The development of the laser device and camera mounting apparatus evolved through diverse stages
188 to produce a robust, precise, accurate and fully adjustable setup.

189 Validation of length estimates obtained by the lasers was performed by comparing the real
190 size of fish to the indirect size estimates. The experiment was conducted on a sample of three dead
191 BFT of different sizes (fork length = 127, 150 and 233 cm). A set of video frames was collected,
192 considering two explorative variables: (a) angle of laser beam (0° , i.e. perpendicular to fish body, 20°
193 and 60°) and (b) distance from the specimen (1 m, 3 m or 5 m).

194 Three video frames for each size, angle and shooting distance were collected as replicates for
195 the image analysis (Fig. 3). The fork length (FL) of each target fish was estimated using laser dots
196 spotted on the tuna body (dot inter-distance = 9 cm) as reference scale. Frames were captured in full
197 HD resolution (1080 pixels; 60 frame per second; GoPro Hero3 Black). Post processing of video
198 frames was performed using Tpsdig2 (Rohlf, 2009).

199 The relative error of FL was calculated by subtracting the estimated length from the actual fish
200 length. Negative values represented underestimates, and positive numbers represented overestimates.
201 The coefficient of variation (CV) was calculated by dividing the standard deviation of the estimate
202 by the mean value and was expressed as a percentage (Thresher and Gunn, 1986). CV is a useful
203 measure of precision that is widely used in field research (Thresher and Gunn, 1986). The standard
204 error (SE) of the measurements was also calculated.

205

206 2.4 . *Tagging operation by traditional 'tonnara' traps*

207

208 During the 2014 fishing season, a tagging campaign was planned based on the framework described
209 above. Tagging operations were planned based on BFT remaining in the trap after the tuna quota was
210 achieved by the fishery (165 metric tonnes for the Sardinian trap).

211 Tagging operations consisted of moving and separating the school of tunas from the *camera* to the
212 *camera della morte*. This was achieved by a sequence of opening/closing of the doors/chambers
213 operated by the fishing team on-board and the SCUBA divers. The net-floor 'corpus' was
214 progressively raised to ~7 m from the sea surface, permitting the fish to swim freely and divers to
215 operate in safe conditions. Tagging was carried out by two free divers using a pneumatic speargun
216 with the equipment described above. To prevent possible post-release infection, each applicator was
217 treated with a waterproof disinfectant spray. Tagging was recorded at all phases by the camera

218 mounted on the speargun (Fig. 4). Length estimation was performed by image analysis, as described
219 above.

220 In order to evaluate the effect of the tagger (the free diver responsible for tagging) on the Correct
221 (C) or Wrong (W) deployment of the tag in the target area (Fig. 2), we analysed video recordings for
222 each tagged BFT. The tag was considered to be in the C when it was inserted in the target area (Cort
223 et al., 2010), whereas all of the other positions were considered Wrong. The effect of the tagger (A
224 or B) on tag position (C or W) was evaluated by a χ^2 test ($\alpha = 0.05$) using the contingency tables for
225 small numbers (Yates, 1934).

226 Fish mortality was also evaluated during tagging operations in the trap. It was evaluated by
227 counting tuna dead over time (minutes) and categorizing them as follows: post-tagging mortality
228 (Post), number of deaths caused by injuries from tag insertion; entanglement mortality (Ent), the
229 number of fish entangled in the trap nets due to swimming exhaustion; and total mortality (Tot), the
230 cumulative mortality of Post and Ent. Once the tagging activities were concluded, the entrapped fish
231 were monitored for 2 h and then released.

232

233 **3. Results**

234

235 *3.1. Penetration test by pneumatic spearguns*

236

237 A total of 36 shots were performed for the explorative variables: shooting distance, speargun length,
238 applicator and BFT size. The shooting distance level of 5 metres was not considered in the statistical
239 analysis because for all shots, the tag did not reach the tuna (NR = 100%). Regardless of applicator
240 type (I vs. Y) and speargun size (85, 97 and 110 cm), statistical analysis showed no significant
241 differences ($P = 0.64$). Significant differences were recorded between shooting distances of 1 m and
242 3 m ($P = 0.0042$). Statistical differences were found between BFT size ($P = 0.0006$) when most of
243 the shots were placed in CP for the small fish (Table 1).

244

245 *3.2. Length validation by laser measurements*

246

247 The image analysis always underestimated the actual fish length (Fig. 5). At 1 m distance, the mean
248 error (\pm SE) was -30.8 ± 26.4 cm (CV = 149.8%), -49 ± 18.4 cm (CV = 65.7) and 73.7 ± 19.6 cm
249 (CV = 46.4) at 0° , 20° and 60° , respectively. At 3 m distance, the mean error was -34.3 ± 23.2 cm
250 (CV=118.3), -46.7 ± 16.0 cm (CV=60.1) and -57.7 ± 15.4 cm (CV = 46.7) at 0° , 20° and 60° ,
251 respectively. At 5 m, the mean error was -39.3 ± 27.9 cm (CV = 124.5) when the lasers were

252 perpendicular to the fish (0°), whereas the mean error was not estimated for 20° and 60° because the
253 lasers were not visible at these offset angles.

254

255 3.3. Tagging activities

256

257 During the 2014 fishing season, a total of 163 BFT were entrapped in the *camera della morte* (the
258 ‘death chamber’) and 63 (39% of the total fish) fish were tagged. The size distribution of the tagged
259 fish is reported in Fig. 6. A total of 41 tags (65% of the total) were inserted in C (Table 2). A
260 significant effect of diver skill was observed in the comparison of Wrong and Correct tag positions
261 ($\chi^2 = 6.10$, $df = 1$; $P < 0.05$).

262 A total of four tagged BFT (3.1%) died during the tagging operation (Fig. 7). One tagged BFT
263 died instantly from spinal cord injuries (Post = 1.6%), a symptom that could be directly attributed to
264 the tagging process. At the end of tagging operations, four additional individuals without tags died
265 from entanglement causes (Ent = 2.5%). The entanglement mortality rate (Ent) increased with tagging
266 operational time starting from the fourth hour, with only one BFT dead until three fish were entrapped
267 in the sixth hour (Fig. 7).

268 Among the tagged fish, only one was recaptured (BYP072413) in the Gulf of Lion after 431 d at
269 liberty. The fish was 140 cm in length at the date of tagging and 201 cm at the date of recapture.

270

271 4. Discussion

272

273 Experience with conventional tagging conducted in the Sardinian trap during the 2013 fishing season
274 (Addis et al., 2014) was the practical basis of the current trial. Our aim was to address the main
275 limitations regarding the use of spearguns for tagging and length estimation. During the tagging
276 activities conducted in 2013, we developed a framework to manage the trap gear for tagging and
277 release purposes. The challenge consisted of adapting the processes and the trap equipment to entrap
278 the tunas to be tagged in the *camera della morte* (death chamber) for convenient and stress-free
279 tagging and release of the fish. Based on the expertise of the trap chief (*Rais*) and considering the
280 technical features of the trap chambers, we excluded an *a priori* tagging operation in the ‘Grande’
281 and ‘Bordonaro’ chambers. Due to the large size and depth of these enclosures, tagging operations in
282 the above chambers were unsuitable because BFT become too scattered and scared after the first shots
283 and are thus difficult to approach. In order to simplify the tagging operations, the net floor (*corpus*)
284 of the death chamber was raised to 5–7 meters to minimize the swimming volume and bring the fish

285 close to the surface for tagging. Tagging in the death chamber ensured that the fish experienced
286 minimal physiological stress, far less than they would have with typical on-board tagging.

287

288 *4.1. Penetration test by pneumatic spearguns*

289

290 The results of the penetration test showed that shooting distance and fish size were the main
291 parameters affecting the correct insertion of the tag. The statistical analysis showed that at 1 m it was
292 preferable to use a stopper immediately after the BFIM applicator to avoid deep penetration of the
293 tags. On the other hand, between 1 and 3 m it was possible to shoot the tag without a stopper, which
294 was confirmed by the taggers during the tagging operations. Considering the size of the BFT,
295 spearguns with a length over of 1 m were needed for the correct insertion of the tag. With spearguns
296 over 3 m, tag insertion was ineffective. The major disadvantage of underwater tagging by speargun
297 is the risk of deficient insertion of the anchoring system, which shortens the tag retention time. Many
298 teams prefer to tag the fish onboard: this ensures that the tag has the highest probability of long
299 retention times (Abascal et al., 2016; Aranda et al., 2013; Block et al., 2005; Cort et al., 2010). The
300 main reason is that it offers a more accurate way to put the tag in the appropriate spot, within the
301 pterygiophores of the fish (Cort et al. 2010). However, Aranda et al. (2013) suggested that for
302 electronic tag there were no difference between tagging animals on deck rather than in the water. The
303 maximum and medium retention times achieved in this study were low (151 days and about 38 days,
304 respectively) and do not give strong support to a general statement on tag retention times depending
305 on the technique. Indeed, tag retention is particularly important for electronic tags due to their cost
306 and the fact that a double anchorage is generally preferred and is impossible to achieve with a
307 speargun. This is not as important matter for conventional tagging compared to e-tags as their cost is
308 minimal but could be one of the explanations why only a little amount of tags has been recovered. It
309 would be interesting to discard tag retention as an issue to compare the retention times achieved with
310 conventional and electronic tags deployed underwater or on deck with an appropriate trial in the traps.
311 This will give quantitative information on this issue and the fish does not need to be recaptured to
312 know whether the tag stayed on the fish.

313

314 *4.2. Length validation by laser measurements*

315

316 The length validation was carried out using two-paired-laser photogrammetry with equipment
317 mounted onto a single camera to project points of light onto the dead BFT, which involved taking
318 measurements of body dimensions from photographs. The photogrammetry method is not new and

319 has been used to measure morphometrics on large terrestrial and marine mammals (Barrickman et al.,
320 2015), the dorsal fins of killer whale *Orcinus orca* (Durban and Parsons, 2006), bottlenose dolphin
321 *Tursiops truncatus* (Rowe and Dawson, 2008), small fish at close range (Mueller et al., 2006;
322 Yoshihara, 1997) and the largest ocean fish (Deakos, 2010; Heppell et al., 2012; Rohner et al., 2011).
323 The main advantage of the technique is that it is relatively simple and compact and can be
324 implemented by a single photographer or operator. In contrast, the estimates of fish length were
325 limited due to high error rates and originated from the variable distance between the fish and the
326 reference scale included in the scene (Trobboni and Venerus, 2015). The machine learning approach
327 along with static and mobile devices have been developed for more accurate and precise estimates of
328 fish with mean relative errors <1% (Harvey et al., 2002, 2001; Harvey and Shortis, 1995; Karakulak
329 et al., 2015). However, the reduction of measurement errors comes with increased equipment costs,
330 as it requires two cameras with housings and specialized stereo-photo software (Bouquet, 2008;
331 Deguara et al., 2014).

332 In the present study, length validation was carried out with the aim of finding the relative error
333 and applying a correction factor to the estimates of fish tagged. The results showed that the length
334 estimation of BFT size was more accurate when the lasers were exactly perpendicular to the animal,
335 and that this method always underestimates BFT size. Although the laser beams were maintained in
336 a perpendicular direction and projected onto the target, a relative error of about -30 cm was detected
337 for all distances and sizes of fish considered. This error measurement was probably due to the image
338 distortion caused by light refraction in the camera housing and the wide angle of the camera lens
339 (Deakos, 2010; Swaminathan and Nayar, 1999). Another possible cause of error is non-parallel
340 alignment of the lasers, but that was not the case in this study, because the laser device had calibration
341 screws to adjust and maintain the laser beams at a constant inter-distance. Moreover, the calibration
342 was carried out before the length validation test and the subsequent tagging activities. The observed
343 increasing error, varying the beam angles, was probably caused by parallax error during the tagging
344 activities, which has been detected as a problematic source of error with paired laser photogrammetry.
345 This occurs when laser projections are not perpendicular to the surface of the target to be measured
346 (Durban and Parsons, 2006). In our study, this type of error could exceed 60 cm of error in estimation
347 at a shooting angle of 60°. However, this high angle was not common during the tagging activities,
348 and the video recordings of BFT at other times allowed us to choose the best frames for image
349 analysis.

350

351 *4.3. 2014 Tagging activities and recapture*

352

353 During the 2014 tagging activities in the Sardinian trap, a total of 63 fish were tagged by a crew
354 composed of two free diver taggers and two on-board assistants. The full operations lasted 3.5 h. The
355 numbers of fish tagged per day was comparable to the number of fish tagged in the Western Atlantic,
356 where BFT schools are abundant (Block, 1998). Analysis of the position of the tag showed that most
357 of the fish (65%) were correctly tagged near the second dorsal fin, with significant statistical
358 differences between the two taggers. These differences were caused both by the different levels of
359 expertise of the taggers and the high mobility of the subjects, which was the main source of error.
360 Several shots did not reach the fish, and schools of large numbers of fishes can create confusion in
361 choosing targets for the tagger.

362 Regarding mortality, only one fish died directly as consequence of tagging injuries; it was the first
363 tagged fish of the day and its death was caused by a lethal shot. The entangling mortality occurred
364 after 2 h of tagging activities. At this time, the fish became stressed and changed their behaviour,
365 becoming more scattered and increasing the number of entangling events. After the tagging
366 operations, the fish were left to calm down for 2 h and then released. Tagging directly underwater
367 could avoid or reduce fish stress as opposed to capturing and tagging on board, which has been found
368 to cause some degree of stress and metabolic disruption (Hoyle et al., 2015; Skomal and Chase, 2002).

369 Only one fish (BYP072413) was recaptured in the Gulf of Lion, after 431 d at liberty. The size of
370 the fish estimated by photogrammetry was 140 cm, whereas the FL at the date of recapture was 201
371 cm. The increase in size perfectly matched the age growth curve for the species (Cort et al., 2014).
372 Tags returned from commercial or recreational fisheries in conjunction with tag-recovery and
373 capture-recapture models are the basis for determining many life history parameters (Rooker et al.,
374 2007). Overall recapture rate, pooled across programs and years, rarely exceeds 10% (Rooker et al.,
375 2007). Most of the tagging programs in the Mediterranean Sea, including our tagging activity from
376 traps, were carried out before the spawning season. These programs were very interesting as it will
377 allow to cover the spawning period and identify the spawning areas for the species. However, such
378 period is also just before the purse seine fishing season (end of May, end of June), during which a
379 very large proportion of the total allowable catch is caught (Di Natale et al., 2018). This probably
380 increases drastically the probability of recapture after a short amount of time or after several months,
381 if the fish were kept in cages (the harvesting season starts generally in October). This could be a
382 second explanation why only a little amount of tags has been recovered. In this case, the traps give
383 the opportunity to explore the possibility to tag the fish later by keeping the fish for some time in the
384 trap and released them after the spawning season in order to be caught the next year.

385

386

387 **5. Conclusions**

388

389 In conclusion, underwater tagging of BFT using the traditional trap had some advantages and
390 disadvantages. The trap represents an optimal system for tagging large numbers of BFT with
391 conventional tags in confined waters and could be also useful for implanting electronic tags (Addis
392 et al., 2016b), temperature loggers (Addis et al., 2013b) and direct visual estimation of abundances
393 (Addis et al., 2013c). The proposed method could be beneficial when the goal is to release the fish in
394 healthy or stress-free conditions. It allowed us to determine the fraction of tagged fish that survive
395 after the initial stress of capture, handling, tagging and release by keeping the fish confined for several
396 hours in the death chambers. Tagging in confined water is extremely useful for the observation of
397 fish health, and in the case of electronic tagging, allowed the recovery of the devices (extremely
398 expensive) when a fish died, or a quick pop-up occurred. Tagging fish underwater requires low
399 turbidity (better clear waters), and the success of the tagging can be affected by the expertise of the
400 tagger and the high mobility of BFT. Length estimation by laser pointers (accuracy) is the main issue
401 with the method and could be avoided when a study is focused on fish size or growth. Indeed,
402 according to Cort's growth curve for Bluefin Tuna (Cort et al., 2014), an error of 30 cm would
403 correspond to a year-class difference, which would make the use of the data difficult for a growth
404 analysis although it is possible to estimate the error and to apply a correction factor to the estimated
405 size.

406

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591

592 **List of tables**

593

594 **Table 1**

595 Ballistic tests with three different size of speargun (85, 97, 110), two applicator (I, Y), two size of
 596 BFT (Small, Giant) and three shooting distances (1, 3, 5 m). TD = too deep; DP = deep; CP = correct
 597 position; NP = not penetrate; NR = not reach (arrive). In bracket: shooting number.

Distance		1 m			3 m			5 m		
Spear gun size		85	97	110	85	97	110	85	97	110
BFT size	Applicator Type									
S	I	TD (n=3)	TD (n=3)	TD (n=3)	CP (n=3)	CP (n=3)	CP (n=3)	NR (n=3)	NR (n=3)	NR (n=3)
G	I	DP (n=3)	DP (n=3)	DP (n=3)	NP (n=3)	CP (n=1) NP (n=2)	CP (n=3)	NR (n=3)	NR (n=3)	NR (n=3)
S	Y	CP (n=3)	CP (n=3)	CP (n=3)	CP (n=1) NP (n=2)	CP (n=3)	CP (n=3)	NR (n=3)	NR (n=3)	NR (n=3)
G	Y	CP (n=3)	CP (n=3)	CP (n=3)	NP (n=3)	NP (n=3)	CP (n=1) NP (n=2)	NR (n=3)	NR (n=3)	NR (n=3)

598

599 **Table 2**

600 Results of the trial testing the effect of two divers (A, B) on the position of the tag (Correct, Wrong).

Diver	Wrong	Correct	Total
A	16	15	31
B	6	26	32
TOTAL	22	41	63

601

Figure Captions

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Fig. 1. The trap array in Sardinia (Cort, permission) consists of nylon nets arranged in a tail and five chambers (from east to west): the “Grande” (120 m x 45 m), the “Bordonaro” (50 m x 45 m), the “Bastardo” (45 m x 40 m), the “Camera di ponente” (45 m x 40 m) and the “Camera della morte” (the “death chamber”) (45 m x 30 m). Only the death chamber has a vertical moving net ‘floor’ (corpus) used to pull up bluefin tuna during the “mattanza”. Once entrapped in the first chamber (the “Grande), tunas swim naturally from east to west chambers crossing the doors (a system of vertical nets with a large mesh size). Bluefin tuna unlikely swim in the reverse path, therefore specimens tend to concentrate into western chambers.

Fig. 2. Target area for the conventional tagging of BFT (Cort et al., 2010) and projections of the fixed-distance laser dots.

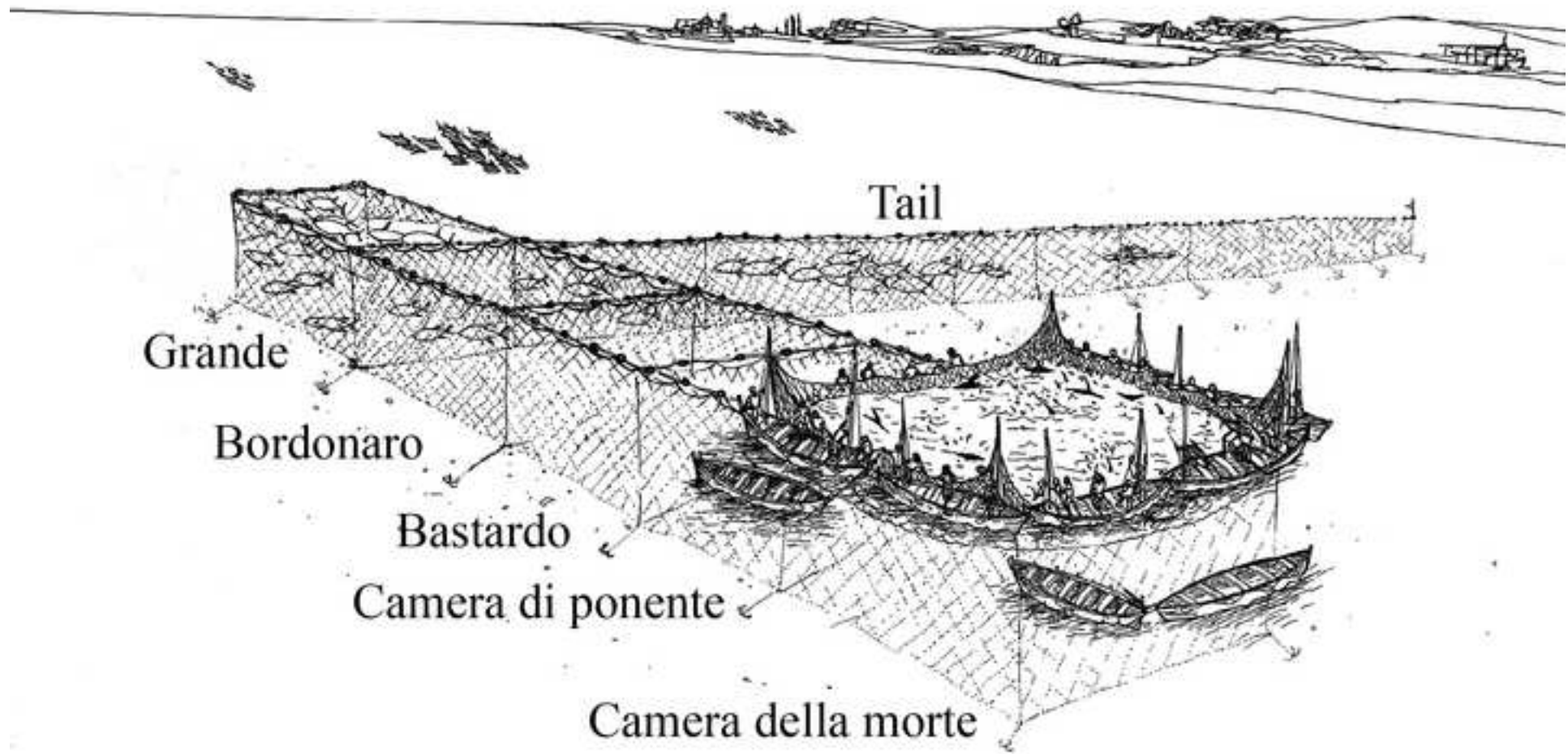
Fig. 3. Setup of the experimental design for length validation using laser measurements. First row: BFT of three different size. Second row: angle of laser beam. Third row: distance (m) from the target. Fourth: replicates of video frames collected for each angle and distance.

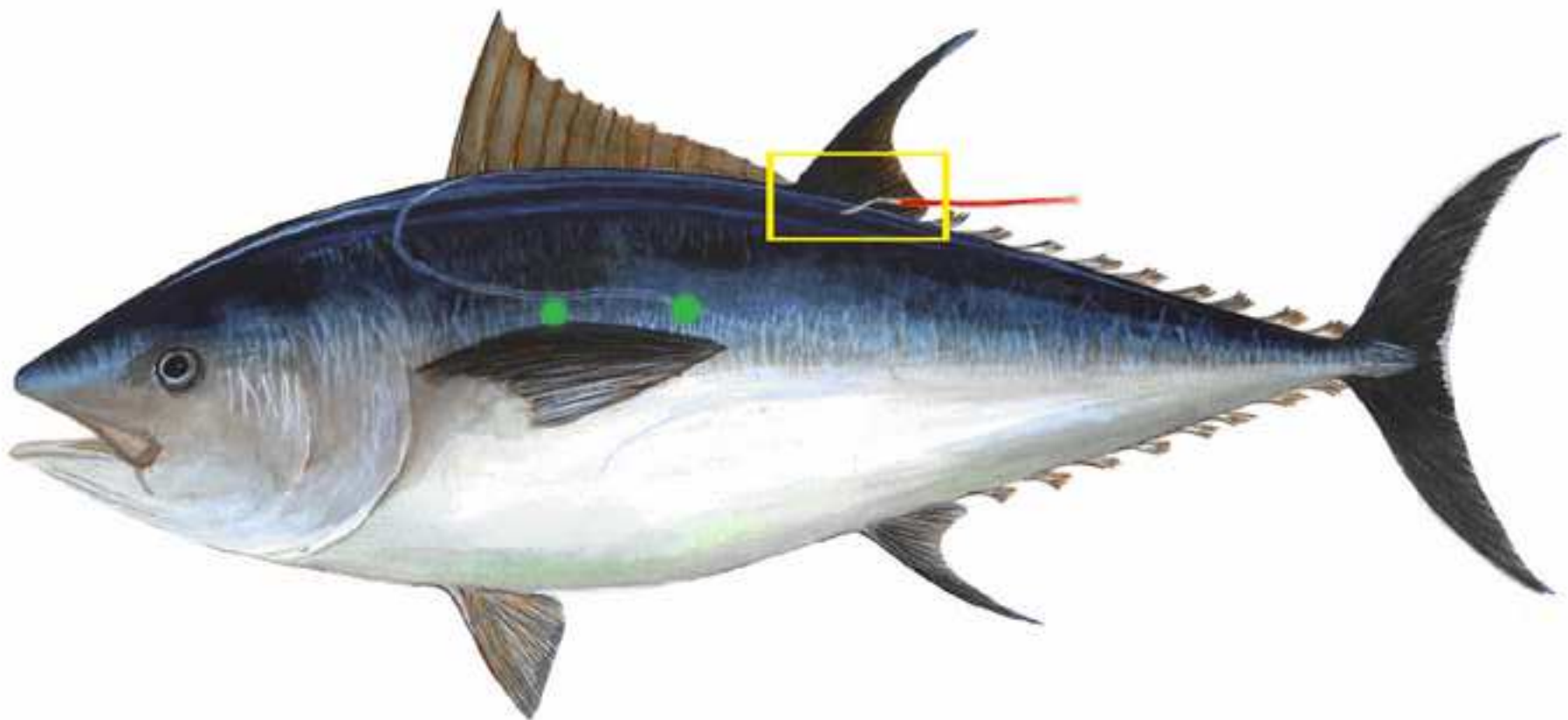
Fig. 4. The tagging phases followed during the 2014 activities: 1) start the video recording; 2) record the number of the tag; 3) Point the fish with the laser 4) tag the fish; 5) ascertain the correct insertion of the tag in the fish, which is the dorsal musculature at the base of the second dorsal fin, and the health of the fish; 6) Release of the fishes.

Fig. 5. The effect of increasing angle of shooting on the accuracy of laser point device in length estimation (Mean error \pm SE and coefficient of variation).

Fig. 6. Bar plot reporting fish tagged for small, medium and giant sizes in the traditional trap of Sardinia in 2014.

Fig. 7. Cumulative number of BFT tagged, cumulative post tagging mortality and cumulative mortality by entanglement during the tagging-time operation in 2014.





Tuna size	S1									S2									S3											
Angle	↑ 0°			↖ 20°			↖ 60°			↑ 0°			↖ 20°			↖ 60°			↑ 0°			↖ 20°			↖ 60°					
Distance	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5
Frames	Three frames									Three frames									Three frames											



