Tagging and Tracking of Marine Animals with Electronic Devices
Reviews: Methods and Technologies in Fish Biology and Fisheries
2009, Volume 9, Part I, Part 3, Pages 181-193
http://dx.doi.org/10.1007/978-1-4020-9640-2_11

The original publication is available at http://www.springerlink.com

Effects of T-bar and DST Tagging on Survival and Growth of European Hake

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Abstract:

Controlled experiments were conducted to assess the effects of T-bar and DST tagging on post-release survival and growth of European hake. In this study, two groups of each 30 hake were considered: small fish (SF, average total length: 29.9 cm ± 2.2 cm) and large fish (LF, average total length: 36.4 cm ± 2.5 cm). Within each size group, fish were randomly assigned to one of 3 treatment groups: control (C), T-bar tagging referred as conventional tagging (CT) and DST tagging (DST) with dummy tags. After 4 months, the overall survival rate was 35%. Smaller fish were less impacted by the stress induced by handling, anaesthesia and tagging and in the SF group, the survival rates were similar (30%) for CT or DST. Specific growth rates were highly variable and no significant difference could be observed between control and tagged fish. Our results demonstrate that (1) conventional tagging affects fish survival rates and (2) DST tagging is feasible in the field on “small fish” with expected survival rate and recapture probability close to that of conventional tagging.

Keywords: *Merluccius merluccius* - Archival tag - Husbandry - Midterm experiment - Captive hake

Introduction

European hake, *Merluccius merluccius*, is a demersal fish widely distributed from the west coast of Norway, south to the coast of Mauritania, and eastwards into the Mediterranean Sea. It is a major demersal resource in the North East Atlantic, which has been heavily exploited by fisheries. Total landings of *M. merluccius* have decreased from 120000t in the early 1960s to 50000t in recent years. Although there is no evidence that multiple populations exist, the northern stock (ICES areas IIIa, IV, VI and VII and VIIIa, b, d) and the southern stock (ICES areas VIIIc and IXa) are managed separately (ICES 2005).

Recent mark-recapture experiments (de Pontual et al. 2003; Pineiro et al. 2007) have provided evidences of growth underestimation of the species due to a bias in the age estimation method internationally agreed and routinely used for the species (de Pontual et al. 2006). Simulations have estimated a significant associated impact on assessment (*e.g.* higher F, lower SSB) and management advices (Bertignac and de Pontual 2007). Besides growth, directed movements and fishing mortality are other key information that can be gained from mark-recapture experiments (Beverton and Holt 1957). To estimate fishing mortality, it is necessary to estimate post release mortality due to the stress of capture, handling and tagging on the fish (Brattey and Cadigan 2004). Estimating post-release mortality is difficult and requires experiments that compare the survival of tagged fish *vs.* control fish held in captivity (Rutecki and Meyers 1992; Millner et al. 1993; Pierce and Tomcko 1993). Furthermore, it is now well recognized that fish tagged with archival tags (also called Data Storage Tags, DST) provide important information about fish movements, behaviour and their environmental conditions (Thorsteinsson 2002). As such internal tagging is more invasive and expensive than external T-bar tagging (referred below as conventional tagging), a feasibility study needed to be conducted before the start of a DST tagging experiment in the field on European hake.

The main challenge to study the effects of conventional tagging on mortality rates in European hake is that this species is not commercially farmed. One reason for this might well be that the species has long been regarded as especially sensitive to capture- and handling-related damages. A pioneering study on larval rearing experiment (Bjelland and Skiftesvik 2006) provided some basic information on egg incubation to weaned larvae. Here we report a study carried out on wild-caught fish kept in captivity. The main objectives were to estimate the effects of conventional and DST tagging on fish survival and growth. The information gained from these research objectives will help determine the feasibility of DST tagging studies at sea.

Methods

Fish origin

Fish were caught in the Bay of Biscay in July 2005 using the capture method developed by de Pontual et al. (2003) for mark-recapture experiments. To acclimatize the fish, individuals were kept in captivity for 7 months in 15 m^3 tanks. These tanks were supplied with seawater flow at ambient temperature (from 7.9°C to 13.7°C), flushed with a daily water renewal rate of 20%, and illuminated following the natural photoperiod. Fish were fed ad libitum on inert preys (sprat, herring and mackerel).

Tagging protocol

Prior to tagging the experimental group of fish (average total length: 31.2 cm ± 2.3 cm and average weight: 190.1 g ± 52 g), we determined the best anaesthesia protocol suitable for further experiments onboard research vessels. Our protocol criteria were the following: low toxicity for both operator and fish, short induction time, short recovery time, small secondary

87 effects, and no withdrawal period. After testing several protocols, we choose Benzocaïne
88 (ethyl *p*-aminobenzoate) at a concentration of 100 mg l^{-1} at a sea water temperature of 9.3°C.
89 The tagging experiment started on February 7, 2006 and ended on June 15, 2006 (128 day
90 duration). Two size groups were selected: small fish (SF, average total length TL ± standard
91 deviation SD: 29.9 cm ± 2.17 cm, N= 30) and large fish (LF, average total length TL 36.4 cm
92 ± 2.5 cm, N=30). Within each size group, fish were randomly assigned to 3 treatment groups:
93 control (C), conventional tagging (CT) and DST tagging (DST).
94 All fish were anaesthetised, measured (total length TL_0) and weighed (W_0) prior to
95 subsequent treatment. SF individuals were exposed to a 100 mg l^{-1} benzocaïne solution, a
96 concentration which was increased to 120 mg l^{-1} for the LF group. Total exposure time to
97 benzocaïne did not vary between groups and was set at 15 min to allow for the time needed to
98 insert DST tags (5 min). Individual fish behaviour such as loss of reaction to external stimuli
99 and loss of equilibrium, was followed during the anaesthesia steps. Conventional tagging (CT
100 group) was carried out according to the method developed by de Pontual et al. (2003) for
101 mark recapture experiment. Briefly, a T-bar tag was inserted at the base and in front of the
102 second dorsal fin and the fish was injected with a solution of oxytetracycline at a dose of 60
103 mg kg^{-1} of fish. This antibiotic is used to mark calcified structures for age validation (de
104 Pontual et al. 2006). In addition, the DST fish group had Star-Oddi DST micro dummy tags
105 (8.3 mm diameter x 25.4 mm length, 3.7 g in air), inserted in the peritoneal cavity. This
106 surgerical procedure consisted of 1) 1 cm cut through the ventral muscle at 1 cm in front of
107 the anus, 2) pushing the tag gently inside the peritoneal cavity and 3) suturing the wound by
108 2-3 stitches with polyamide monofilament (Bégout Anras et al. 2003). After treatment, fish
109 were transferred to two 15 m^3 indoor tanks, one for the SF group (thus including 3 groups:
110 SF-C, SF-CT, SF-DST) and the second for the LF group (LF-C, LF-CT, LF-DST). At the end
111 of the experiment fish were killed with an overdose of anaesthetic and measured (total length

TL_f), weighed (W_f) and sexed. Euthanized and fish dead during the course of the experiment were deep frozen prior subsequent treatment.

Data analyses

The condition factor of individual fish was calculated as (Wege and Anderson 1978; Jobling et al. 2001):

$$K_t = W_t/W_e x100 \quad \text{(Eqn 1)}$$

where W_t is the weight of the fish at time t and W_e is the theoretical weight calculated from the length-weight relationship derived from the field: W_e (g) = 0.00513 $L^{3.074}$ (Dorel 1986). Specific Growth Rate (SGR % day^{-1}) was calculated as:

$$SGR = (e^g-1)\ x100 \quad \text{(Eqn 2)}$$

where $g = [\ln(W_{tf})-\ln(W_{t0})]/(t_f-t_0)\ ]$ and W_{tf} and W_{t0} are the wet body weight (g) at death or experimental end (t_f) and tagging (t_0) time respectively (Houde and Schekter 1981; Nordgarden et al. 2003). Estimates of individual growth rate were not available for the control groups (SF_C, LF_C) as, by definition, fish could not be tagged. We addressed the later issue by considering average SGRs for both SF_C and LF_C groups estimated from the total mass growth with respect to the total survival time. For the 20 fish that had DST implantations, we analysed the impact of insertion on mean survival. The tag to fish weight ratio (R) was calculated as:

$$R = W_{DST}/W_0*100 \quad \text{(Eqn 3)}$$

where W_{DST} is the DST weight in air and W_0 the fish wet weight. Two groups were considered: R1 with R <2% and R2 with R $\geq$ 2% (Winter 1983).

Measurements of length, weight and SGR expressed as average ± standard deviation (SD), were compared using ANOVA or t-tests with treatments and group size as factors after data had been tested for normality. Estimates of condition factors were compared using Kruskal-Wallis test (KW). Survival analysis was conducted using Kaplan-Meier analysis with respect to treatment and group size. However, as the initial condition (K_0) impacts fish survival, we also applied Cox regression analysis with K_0 as covariable. Spearman's rank correlation was used to test the relationship between an individual's initial condition factor and survival rate. Statistical analyses were performed using SPSS 14.0. (SPSS, USA) and the significance level was set at $P < 0.05$.

Results

Analysis of initial fish characteristics according to treatment and group size

At the end of the acclimation period, 68% of fish had an initial condition factor (Kt0) greater than 100%. Thus, we considered that acclimazation to rearing conditions had been achieved for most individuals after 7 months. At the beginning of the experiment, Kt0 ranged from 71% to 127% and three Kt0 classes (<80%, 80-100% and >100%) were considered for each group and treatment (Table 1). The initial condition factor did not differ (a) between groups (Table 1, KW Test: df = 1, P= 0.906), or b) between treatments (Table 1, KW Test: df = 2, P= 0.400).

[Table 1 here]

Effect of tagging on survival

Out of the initial 60 fish, 21 were survived until the end of the experiment (day 128), which corresponds to an overall survival rate of 35%. Kaplan-Meier survival functions with respect to treatments (C, CT and DST) and size groups (SF and LF) showed that, regardless of fish size, tagging (CT or DST) severely decreased survival probability compared to the control group (Figure 1). Mortality was observed 50 days after tagging and after this time period mortality stabilized in at least the SF group. It is worth noting that, for both SF and LF groups, survival proportion did not differ significantly between CT and DST groups (Log Rank tests: SF: $P_{C/CT}$=0.017, $P_{C/DST}$=0.07, $P_{CT/DST}$=0.777; LF: $P_{C/CT}$=0.021, $P_{C/DST}$=0.003, $P_{CT/DST}$=0.705). Moreover, it is worth noting that no mortality has been observed in anaesthetized groups during the preliminary experiment after 24 days.

[Figure 1 here]

The initial condition Kt0 had a clear effect on the survival rate of fish. The seven tagged fish that had a Kt0<80, all died within the first 28 days. The correlation between survival time and Kt0 was significant when all tagged fish were considered (Spearman correlation test, N = 40, P= 0.006), whereas no significant correlation was observed if fish with a Kt0≤80 were removed from the statistical analysis. Cox regression analysis (Figure 2) indicated that DST tagging may slightly decrease the survival probability compared to conventional tagging.

[Figure 2 here]

The R ratio also affected survival rate. Survival differed significantly between R1 and R2 (Log Rank tests: $P_{R1/R2}$=0.023) and actually, all fish with R>2% died rapidly after tagging (Figure 3).

[Figure 3 here]

Effect of tagging on growth

Determining the effects of tagging on fish growth was limited by two factors. First, survival at the end of the experiment was low for tagged groups (CT, DST), especially for the LF group (Figure 1). Second, the control fish were not tagged. The total masses of SF_C group were 1804.5 g and 2813.0 g at the start and the end of experiment, respectively. The survival time for this group was 1138 days and the SGR was estimated at 0.039 % day^{-1}. The corresponding data for the LF_C group was respectively 3428 g, 4066 g and 936 days resulting in a SRG of 0.018 % day^{-1}.

[Table 2 here]

Statistical analysis of the tagged groups revealed relatively high individual growth variability regardless of the size and treatment group (Table 2). Negative SGR were observed for fish that had a very poor initial condition and died rapidly after tagging (Figure 4a). It is worth noting that one fish in poor initial condition survived until the end of the experiment (Figure 4). Comparisons of SGR between control and treatment fish showed that tagging did not significantly affect the SGR (t-tests, df = 9, P_{SF_CT} =0.145, P_{SF_DST} =0.117, P_{LF_CT} =0.197, P_{LF_DST} =0.346).

Progressive feeding resumption occurred after 7 days post tagging. It started at a low level (0.5 prey day^{-1} per fish) and then increased to 1 prey day^{-1} per fish. The first week post tagging could thus be considered as a critical period characterized by fasting and death of fish that had poor initial condition.

212

213 [Figure 4 here]

214

215 **Discussion**

216 **Effect of tagging on survival**

217 Anaesthetics doses applied to European hake during our experiments (100-120 mg l^{-1}),
218 correspond to the upper limit of those reported for other species (Soivio et al. 1977; Iwama et
219 al. 1989; Iversen et al. 2003). Considering the required doses and deep anaesthesia induction
220 time, hake seems to be relatively resistant to anaesthesia.

221 The first fact to be considered is that handling and anaesthesia might well be more harmful
222 than expected from the short term (24 days) preliminary experiment as the mortality rate
223 reached 30% in control group. The different mortality rates observed in the LF and SF groups
224 also suggests that the former is more sensitive (40% against 20% for LF and SF respectively).
225 We hypothesize that poor initial condition is a factor limiting survival for some fish, although
226 direct evidence is missing because control fish were not individualized. The low ambient
227 temperature (9°C) at the beginning of the experiment may have also been partly responsible
228 for difficult recovery. Actually subsequent pilot tests have demonstrated that winter is not the
229 best period for supplying experimental facilities with wild hake (de Pontual et al. unpublished
230 data).

231 In terms of conventional tagging, survival rate was 30% for small tagged fish against 70% for
232 the control group. These results confirm the observations made on controls regarding the
233 higher ability of small fish to resist to physical stress. These results also indicate that, the CT
234 tagging process has a strong effect on survival probability. This could be explained both by
235 species-specific response and a sub-optimal tagging protocols. However, several studies have
236 demonstrated negative effects of tag application to wild and hatchery fish on survival, in

particular on salmon (Saunders and Allen 1967; Isaksson and Bergman 1978; Hansen 1988; Moffett et al. 1997; Crozier and Kennedy 2002). Another important consideration is the duration of the experiment. Two periods can be distinguished in terms of the fish mortality rates. The first mortality phase extended to about 50 days post tagging (handling and tagging effects), whereas the second phase occurred at the end of the experiment (fish probably died due to nutritional stress because of unsuccessful feeding resumption, see below). Such a result questions the reliability of short term experiments, which may well provide biased estimations of mortality. This is the case for very short term (2-5 days) experiments held on research vessel during tagging surveys (*e.g.* de Pontual et al. 2003 for European hake). This issue has also been emphasised for short term (5-10 days) experiments in submersible enclosures (Brattey and Cadigan 2004). To best estimate port-release mortality rates for tagged fish, individual fish must be observed for longer periods of time.

A higher mortality rate in DST tagged fish than CT fish could result from the invasive surgery. Surprisingly survival probabilities in DST and CT fish were similar at least in small fish. The removal of a probable Kt_0 effect only slightly decreased the survival probability. However, the tag to body weight ratio (R) has an effect on hake survival. This is in accordance with earlier work showing higher mortality and/or reduced swimming performance in DST-tagged fish (Marty and Summerfelt 1986; Greenstreet and Morgan 1989; Peake et al. 1997; Adams et al. 1998). Our results confirm that this ratio should not be greater than 2% (Stasko and Pincock 1977; Winter 1983) even if the question is challenged (Jepsen et al. 2005). Based on the hake length-weight relationship (Dorel 1986), Star-Oddi DST micro tags should not be placed on a body wet weight less than 180 g, which corresponds to a total length of approximately 30 cm.

Effect of tagging on growth

Food resumption started only 7 days post-tagging and progressively increased after this period. Food consumption remained low relative to fish in other stocking tanks until c.a. 100 days post tagging. Consequently, we can hypothesize that fish first experienced a weight loss phase due to fasting. This assumption is supported by a strong negative SGR of fish that died early in the experiment. The recovery process may have been longer than in nature as mark-recapture results indicated that fish stopped growing for 20-50 days after release (de Pontual et al. 2006). This might relate to upset feeding behaviour as hake acclimation on inert preys had proved to be a challenging process. It may also explain the difference observed in the growth rates estimated in this study (0.013 ± 0.016 cm d^{-1}) and estimates obtained from field experiment (up to 0.054 ± 0.004 cm d^{-1} for fish which had one year or more at liberty; de Pontual et al. 2006). An important outcome of the present work is that growth did not differ significantly between control and tagged fish. This result corroborates the findings of previous works on species such as European sea bass (Bégout Anras et al. 2003), juvenile cod (Jensen 1967; Tranquilli and Childers 1982; Svåsand et al. 1990; Cote et al. 1999), and adult cod (Righton et al. 2006). The latter concluded that tagging had no long term effect on growth except on the gonads mass, where tags could potentially occupy the space for gonad growth (Righton et al. 2006).

Conclusion

In this study, the first estimates of post tagging mortality were established and these results suggest mortality after tagging might be high in field experiments. They have to be refined before mark-recapture data can be used to estimate exploitation rates and population sizes. Our results also suggest that improvements in tag implantation could increase post tagging survival rate. Contrary to initial predictions, similar survival rates were observed for fish tagged with DST and conventional tags. Recovery rate close to that of conventional tagging

can reasonably be expected. Results also emphasize the need for a thorough fish selection prior to DST tagging based on fish size and initial condition. Small fish with a high initial condition would be the most suitable for future tagging studies in the field. Actually, criteria derived from this experiment have provided suitable basis for a successful field pilot study which analysis is ongoing (de Pontual et al. unpublished data).

Acknowledgements

The authors are thankful to M. Suquet for his help during experiment and fruitful discussion on hake rearing. Furthermore, we thank H. Arrizabalaga, N. Jepsen, I. Quincoces and an anonymous referee for their reviews and B. Graham for improving the language. This study was conducted under the approval of the Animal Care Committee of France under the official licence of M.L. Bégout (17-010).

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Table 1: Percentage of fish with an initial condition factor (Kt0) in each size group and the average value of Kt_0 (%) (± standard deviation SD %) with respect to each size group (SF and LF) and treatment group (C: control, CT: conventional tag, DST: data storage tag).

			Group		
			Small fish	Large fish	Total
Treatment	control	Kt0 < 80%	0	20	10
		Kt0 80-100%	10	20	15
		Kt0 >100%	90	60	75
		Total	**100**	**100**	**100**
		Average Kt0 ± SD	105.2 (± 6.7)	100.3 (±15.5)	102.7 (±11.9)
	CT	Kt0 < 80%	10	30	20
		Kt0 80-100%	30	10	20
		Kt0 >100%	60	60	60
		Total	**100**	**100**	**100**
		Average Kt0 ± SD	99.2 (± 11.9)	96.1 (± 16.7)	97.7 (± 14.2)
	DST	Kt0 < 80%	20	10	15
		Kt0 80-100%	10	20	15
		Kt0 >100%	70	70	70
		Total	**100**	**100**	**100**
		Average Kt0 ± SD	100.3 (± 15.2)	102.9 (± 14.7)	101.6 (± 14.6)
		Average Kt0 ± SD	101.6 ± 11.7	99.7 (± 15.4)	

Table 2: Growth characteristics with respect to size group (SF and LF) and treatment group (CT: conventional tag, DST: data storage tag). SGR: specific growth rate (in % $days^{-1}$), W_{t0}: initial weight (g) and W_{tf}: final weight (g).

Group _ treatment		SGR	W_{t0}	W_{tf}
SF_CT	N	10	10	10
	Mean	-1.7	174.6	181.9
	Standard deviation	1.1	11.6	12.9
	Minimum	-9.7	95	129
	Maximum	0.8	240	248
SF_DST	N	10	10	10
	Mean	-0.7	195	214.6
	Standard deviation	0.5	13.9	28.8
	Minimum	-4.1	110	97
	Maximum	0.7	256	443
LF_CT	N	10	10	10
	Mean	-0.3	314.7	304.2
	Standard deviation	0.3	21	23.8
	Minimum	-2.6	212	196
	Maximum	0.2	423	415
LF_DST	N	10	10	10
	Mean	-0.1	325.1	320.7
	Standard deviation	0.1	27.8	32.3
	Minimum	-0.6	196	190
	Maximum	0.2	503	520
Total	N	40	40	40
	Mean	-0.7	252.4	255.4
	Standard deviation	0.3	14.4	15.4
	Minimum	-9.7	95	97
	Maximum	0.8	503	520

Figure legends

Figure 1: Kaplan Meier survival functions for (A) small fish (SF) and (B) large fish (LF). Control (dotted line), Conventional tagging (dashed line), DST tagging (solid line).

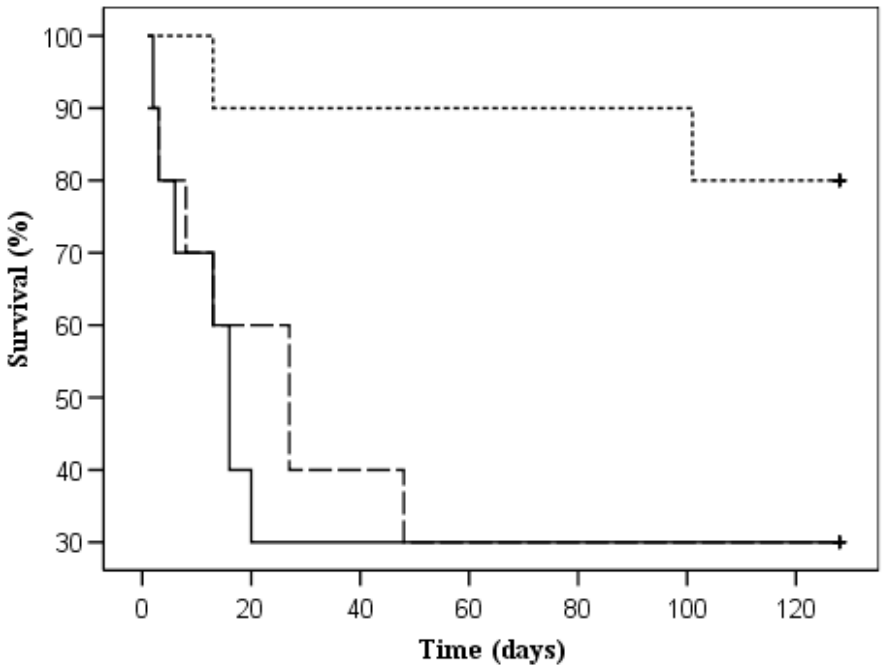
Figure 2: Survival functions with respect to fish size and tagging method derived from the Cox regression with initial condition factor as a covariate. Small Fish – Conventional Tagging (SF-CT, solid line), Small Fish – DST Tagging (SF-DST, dotted line), Large Fish – Conventional Tagging (LF-CT, dashed line), Large Fish – DST Tagging (LF-DST, dash-dotted line).

Figure 3: Survival functions of DST tagged fish for different tag to fish weight ratios (R) derived from Cox regression with initial condition factor as covariate. R<2% (solid line), R≥2% (dotted line).

Figure 4: Time distribution of Specific Growth Rates (SGR) estimated at fish death for different fish size and tagging methods: Small Fish – Conventional Tagging (SF-CT, Δ), Small Fish – DST Tagging (SF-DST,), Large Fish – Conventional Tagging (LF-CT, ×), Large Fish – DST Tagging (LF-DST, ○). SGR individual values are specified for fish that had a particularly poor initial condition.

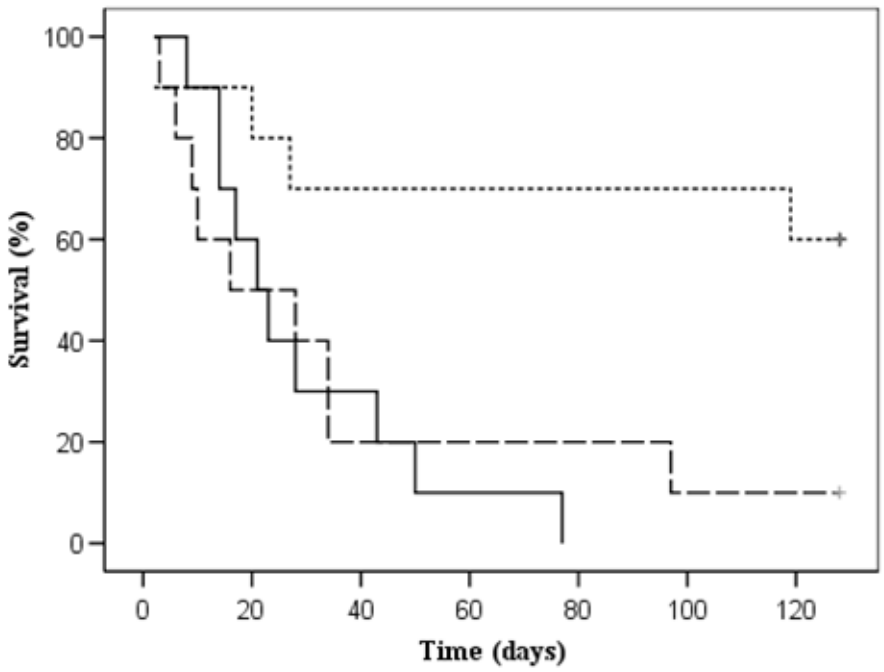
435 **Figure 1:**

436 (A)



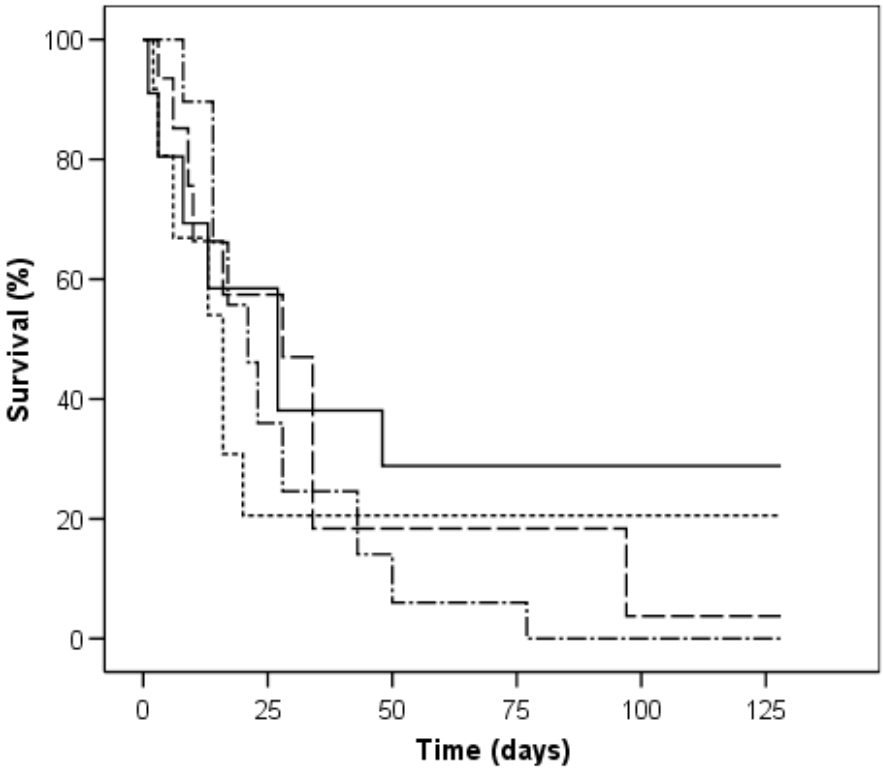
437

438 (B)

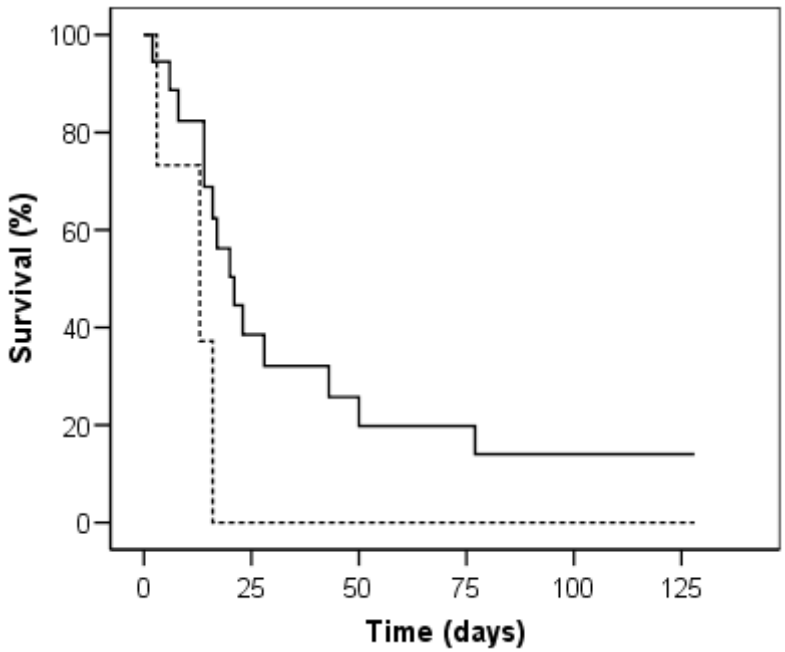


439

Figure 2:

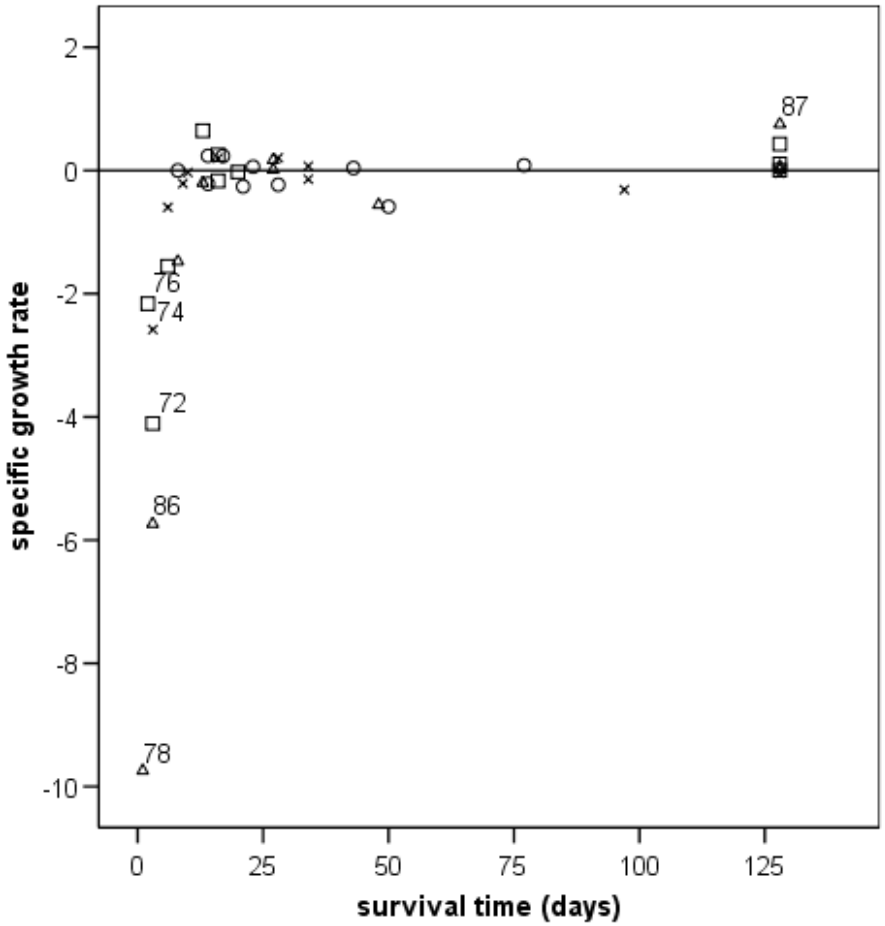


442 **Figure 3:**



443

444 **Figure 4**:



445