Cruise Report
ZDLK3-10-2023

Patagonian toothfish (*Dissostichus eleginoides*) tagging and gonad sampling

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November 2023

ZDLK3 - 10 - 2023

DESIRE THE RIGHT

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Acknowledgements

We thank Consolidated Fisheries Ltd. for their continued assistance and support, the Captain, the officers and the crew of the *CFL Hunter* for their hospitality and cooperation during this trip at sea.

For citation purposes this publication should be referenced as follows:

Le Luherne E, Peruzzo M. 2023. Cruise Report ZDLK3-10-2023: Patagonian toothfish (*Dissostichus eleginoides*) tagging trip. Fisheries Department, Directorate of Natural Resources, Falkland Islands Government, Stanley, Falkland Islands. 14 p.

Distribution: Public Domain

Reviewed and approved by:

Andrea Clausen Date: 14/12/2023
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Summary

The research cruise ZDLK3-10-2023 focused on Patagonian toothfish (*Dissostichus eleginoides*, hereafter toothfish) and was conducted onboard the *CFL Hunter* (ZDLK3) between 19th October and 2nd November 2023 within the Falkland Islands waters. Toothfish fishing area was divided into three sub-areas, North (above the -50°S), East and South (below the -53.3°S) according to habitat characteristics. The objectives of the research cruise were (1) to tag 800-1000 toothfish and (2) to sample toothfish gonads. Routine scientific observation protocols were also performed, as detailed in Observer Report 1383 (FIFD, 2023a). We tagged 878 toothfish using external spaghetti Floy® dart tags (73 fish tagged per day on average) and we sampled the gonads of 107 toothfish. The total amount of toothfish tagged corresponds to 23% of the total catches in terms of both number and weight. Of these, 325 toothfish were tagged in the North, 241 in the East and 312 in the South. The total length of the tagged toothfish ranged from 52 to 170 cm (mean ± sd: 101 ± 17.1 cm). We sampled the gonads of 62 females and 45 males, which were mainly macroscopically identified to belong to stages 2 and 3. Among these, 31 were sampled in the North, 19 in the East and 57 in the South. The total length of the toothfish sampled for gonads ranged from 71 to 184 cm (mean ± sd: 119 ± 26.5 cm).

1. Introduction

The tagging programme for the Falkland Islands toothfish was initiated in 2016, aiming to improve our understanding of toothfish movement patterns within the region. The initial goal of tagging 3000 fish was achieved during four tagging research cruises onboard the longliner in 2016-2018 (Randhawa and Lee 2016, Randhawa *et al.* 2017, Farrugia and Keningale 2018, Farrugia *et al.* 2018). In addition to surveys, Scientific Fisheries Observers have been tasked to tag an average of 25 toothfish per week during their trips onboard the longliner. However, the tagging programme has largely been reliant on dedicated research cruises; in their absence, the number of tagged toothfish declined considerably in 2019-2020. In response, a 4-year extension of the tagging programme has been recommended (Lee and Skeljo 2020) and followed up by renewed tagging efforts in 2021 and 2022 (Skeljo and Pearman 2021; Nicholls and Raczynski, 2023) with a goal of tagging ~1000 longline-caught fish annually, i.e. one fish per tonne of TAC.

Since the tagging programme's inception in 2016, specific protocols have been added to the tagging cruises to improve our knowledge of the Falkland Islands toothfish fishery (e.g. deployment of the underwater camera to assess habitat biodiversity and the impacts of umbrella-system longline fishing on habitats in 2017-2020, or sampling of benthic invertebrate bycatch to compile a reference fauna collection in 2020). In the current cruise, the additional protocol was to sample toothfish gonads. Gaps in our knowledge of toothfish reproductive strategy have been highlighted in the annual stock assessment reports (Skeljo *et al*. 2022, 2023). The gonad samples were thus collected to help obtain reliable estimates of toothfish reproductive traits, e.g. age at first maturity, fecundity, and prevalence of skipped spawning in females.

The research cruise was conducted onboard the *CFL Hunter* (ZDLK3), registered in the Falkland Islands. The aims of the survey were (1) to tag a minimum of 800-1000 Patagonian toothfish to meet the annual objectives of the toothfish tag-recapture programme, (2) to sample toothfish gonads to improve our knowledge of toothfish reproductive strategy, and (3) to collect catch composition and biological data from the toothfish longline fishery according to routine scientific observer protocols.

2. Materials and methods

2.1. Cruise itinerary and setting

The research cruise lasted 15 days from 19th October to 2nd November 2023, with 12 days of effective fishing. The 19th October and 2nd November 2023 were respectively allocated to the steaming to the

first station and to Stanley, and on 30th October, the weather did not allow us to fish. The fishing area was divided into three sub-areas, North, East and South according to habitat characteristics. Within the fishing area, the latitude -50 delineated North and East sub-areas and the latitude -53.3 delineated East and South sub-areas. We planned to tag toothfish in the same proportion in the three areas.

The *CFL Hunter* (ZDLK3) departed Stanley and steamed East to set the first lines (stations 1564-1565; Figure 1). From 20th to 24th October 2023, we followed the route set for the cruise to the northern stations (stations 1566-1572; Figure 1). On 24th October the vessel steamed back to the eastern stations (1573-1577) and then to the southern stations (1578-1583) on 28th October 2023 (Figure 1). Sperm whales (*Physeter macrocephalus*) were sighted near the vessel on 28th October 2023. The tagging was interrupted to avoid released toothfish being consumed by the sperm whales and to not reinforce an associated depredation behaviour.

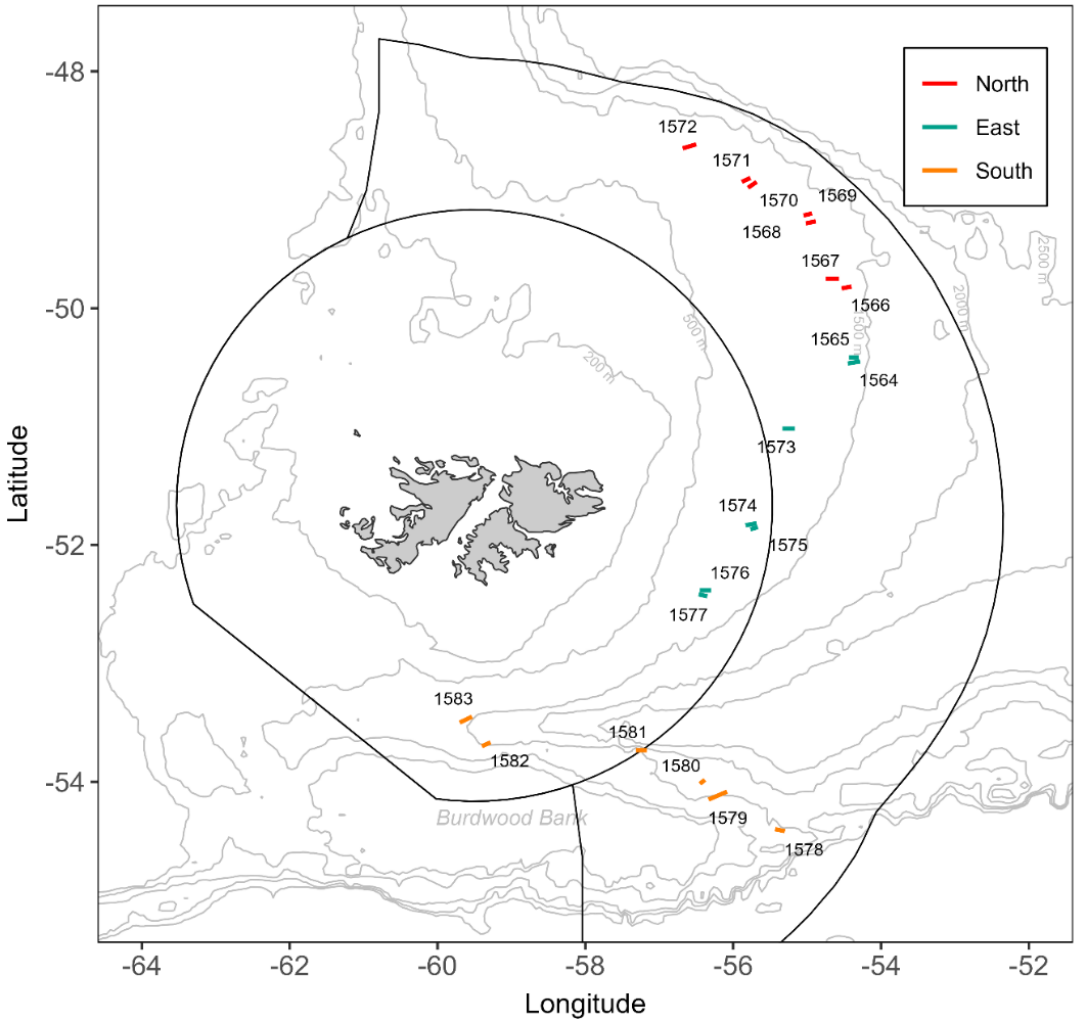

Figure 1. Location of the 20 stations and their corresponding sub-area (North, East and South) of the ZDLK3-10-2023 research cruise. Within the fishing area, the latitude -50 delineated North and East sub-areas and the latitude -53.3 delineated East and south sub-areas.

In total, tagging was conducted on 20 lines during the cruise: 7 in the North, 7 in the East, and 6 in the South (Table 1). We hauled 2 lines per day during the daytime, except for 4 days when only one line was set due to the weather conditions or a long steaming time to the next station. Lines were set between 21:00 and 06:30. The first haul started between 06:50 and 07:40, and the second between 12:00 and 14:30 (Table 1). The soak time was calculated as the difference between the mid-set and mid-haul times. The mean soak time of the cruise was 11:45 ± 2:05 h (i.e., mean ± standard deviation; corresponding to 705 ± 125 min; Table 1). The soak time ranged from 8:28 to 15:50 h (corresponding to 508 – 950 min; Table 1). Station depth was calculated as the mean between haul start and haul end depths. The average station depth was 1307 ± 202 m (Table 1). Tagging was conducted at each station and was prioritised during the first daily station, monitoring the entire line. The second daily station was used to reach the target number of tagged fish (50-70 toothfish per day). Gonads were sampled at 13 stations (Table 1).

Table 1. Station characteristics: sub-area (North, East and South), date, time at haul start, station depth (mean and standard deviation), soak time, and sampling type (T= Tagging, G_R= Gonad sampling random and G_N= Gonad sampling non-random). Station depth was calculated as the mean between haul start and haul end depths. Soak time was calculated as the difference between the mid-set and mid-haul times. Stations are sorted by area and haul date.

Area	Station	Date	Haul start	Depth mean	Soak time	Sampling
North	1566	21/10/2023	06:51	1265 ± 35	10:07	T
	1567	21/10/2023	13:14	1210 ± 42	13:41	T
	1568	22/10/2023	06:49	1170 ± 71	11:24	T/G_R
	1569	22/10/2023	13:20	1184 ± 58	15:50	T
	1570	23/10/2023	07:00	1058 ± 39	11:08	T
	1571	23/10/2023	13:08	1108 ± 2	10:34	T/G_R
	1572	24/10/2023	07:01	1116 ± 12	11:41	T/G_R
East	1564	20/10/2023	06:47	1432 ± 46	10:23	T
	1565	20/10/2023	14:28	1408 ± 32	14:53	T
	1573	25/10/2023	12:05	1188 ± 104	10:34	T/G_N
	1574	26/10/2023	06:57	1142 ± 11	09:34	T/G_R
	1575	26/10/2023	13:00	1190 ± 14	13:13	T
	1576	27/10/2023	06:57	1228 ± 88	09:33	T/G_N
	1577	27/10/2023	13:18	1264 ± 52	12:34	$T/G_{R/N}$
South	1578	28/10/2023	13:40	1325 ± 35	10:11	T/G_R
	1579	29/10/2023	06:55	1548 ± 138	08:28	T/G_N
	1580	29/10/2023	13:25	1722 ± 32	12:13	T/G_N
	1581	31/10/2023	07:40	1590 ± 431	14:34	$T/G_{R/N}$
	1582	01/11/2023	07:03	1535 ± 148	10:07	T/G_R
	1583	01/11/2023	14:23	1462 ± 31	14:32	$T/G_{R/N}$

2.2. Tagging protocol

The tagging station was set up in the dry section of the factory, at the far end of the umbrella cleaning table. The station was prepared with the bolt cutters to remove hooks, a tape measure, the tag board with tags ready to be deployed, the tag applicators, a vial filled with 96% ethanol to disinfect the applicator and the tag before each tagging, and a scribing board. A tag holding board was used, allowing each pair of numbered tags to be prepared in advance in ascending order (usually the night before). Tags were kept together ready to be inserted into the tag applicators. Once a toothfish was brought on board, it was assessed for tagging suitability according to the criteria set by Lee (2022). Tagged fish should match the size distribution of captured fish. The length of the fish is thus not a criterion for fish suitability. However, large toothfish that were lifted onboard without support were not tagged due to the potential for spinal damage. If the weight was supported by the umbrella, then the condition of the fish was considered suitable for tagging. If deemed unsuitable, the fish was forwarded to the factory for processing.

Suitable toothfish were slid from the hauling bay to the tagging station. The remaining hooks were removed using bolt cutters, with minimum injury to the fish. Fish were measured (Total length to the nearest cm) and tagged with one or two external spaghetti Floy® dart tags containing the same unique identification number. At the onset of the toothfish tagging programme in 2016, a series of 6499 paired external spaghetti Floy® dart tags have been procured (*FT*-2-*94 and FT*-1-*94,* small = 75 mm and large = 80 mm, respectively, Floy Tag and Mfg, Inc., Seattle, WA. USA) and used to double-tag toothfish up to 2023. Information on recaptures of double-tagged toothfish has been used to determine the rate of tag loss for both small and large tags (Skeljo *et al*. 2022, in prep.). With tag-loss information obtained, the tagging protocol changed in 2023 to using a single large tag. Given a known tag loss rate, the use of a single tag provides adequate tag-recapture information for stock assessment. Therefore, a series of 10000 single large tags (*FT*-1-*94*, Floy Tag and Mfg, Inc., Seattle, WA. USA) has been procured in 2023. The current cruise has seen the last paired tags deployed (ending with tag pair ID: 6499) and the first single tags deployed (starting with tag ID: 6500).

A sharp hollow applicator was used to insert the tag into the dorsal musculature between the 3rd and 4th rays of the second dorsal fin rays, ensuring that the barb of the tag was locked behind a pterygiophore (Figure 2). Before tagging, applicator and inserted tag were dipped in 96% ethanol to disinfect the material and avoid infection around the tag. The tagged fish were then carried to the hauling bay and returned headfirst into the water. The likely fate of the fish, based on its vigour swimming after release or its attack by predators, along with any notable observations was recorded for future reference.

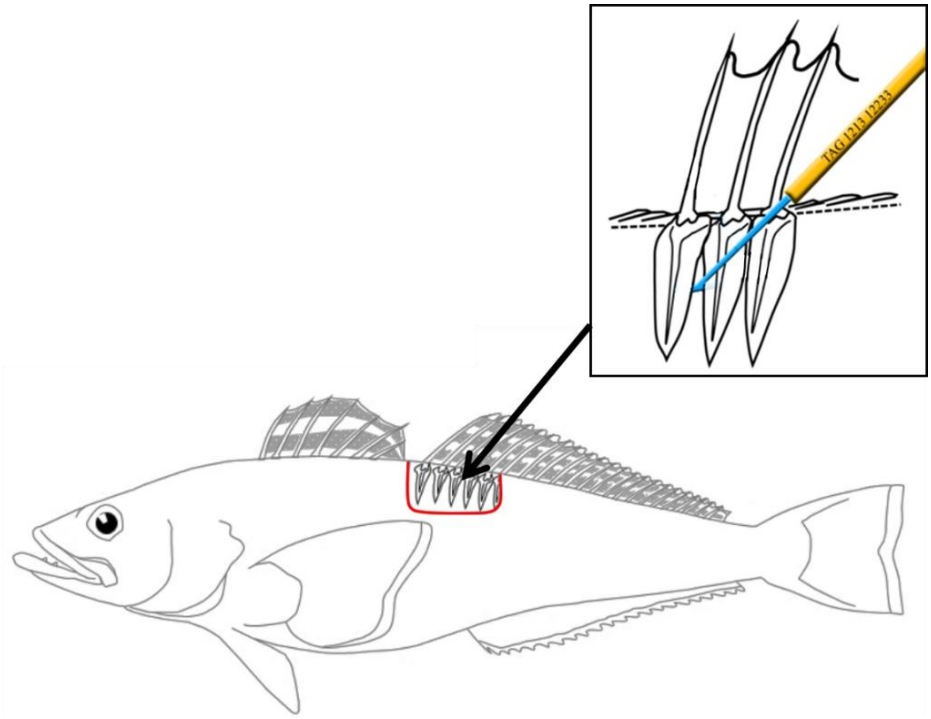

Figure 2. Tagging location between the 3rd and the 4th rays of the second dorsal fin rays locking the barb of the tag behind a pterygiophore. Modified from *Toothfish and skate tagging methods* (CCAMLR, 2013) (draw credits: Alan Hart).

Based on the previous tagging research surveys and the number of toothfish tagged annually by the Scientific Fisheries Observers, we planned to tag between 800 and 1000 toothfish (i.e. between 50 and 70 toothfish per day) and to deploy an equal number of tags between North, East and South sub-areas (Figure 1). At each station/line, we recorded the time when we started and ended to monitor the line for tagging. This information was used (1) to calculate the percentage of the line monitored by the scientist (i.e. the time spent tagging or waiting for suitable toothfish to tag) and (2) to calculate the number of toothfish caught on the portion of the line monitored by the scientist. At the bridge, the time at each marking, and the number of toothfish caught between two markings, were routinely recorded on the logbook. These data were used to calculate the percentage of the line monitored by the scientist and the percentage of tagged fish (in number) during the portion of the line monitored by the scientist. As recommended in Skeljo and Pearman (2021), tagged toothfish were not weighed. Individual weight was calculated using the length-weight relationship set in the database ($W = a L^b$, with a= 0.0061 and b= 3.1037).

2.3. Gonad sampling protocol

Toothfish reproductive strategy sampling was conducted across the entire fishing area. At least 20 fish, representative of macroscopically identified maturity stages, were sampled per sub-area (North, East and South, Figure 1). We also conducted non-random sampling of large toothfish (>140 cm) and toothfish with a maturity stage ≥ 4 (macroscopically identified; 8-stage maturity scale developed by the FIFD; Brickle *et al*. 2006).

The sampling was conducted in the wet factory. The following information were recorded for each sampled fish: total length (to the nearest cm), total weight (to the nearest 100 g), macroscopic maturity stage (8-stage maturity scale developed by the FIFD; Brickle *et al*. 2006), total liver weight (to the nearest g) and total gonad weight (to the nearest g). A small piece of the gonads (~2 cm^2) was sampled, labelled (TOO/Station/Sex_Maturity/indiv_histo_number/otolith_serial_number), and stored in a vial. Otoliths were sampled and stored following the routine observer protocol (FIFD, 2023b) and adding the histological serial number on the envelope. At the end of the station, vials were topped with 10% Buffer Formalin Solution at the dry factory (i.e., not at the sampling factory), the caps were sealed with Parafilm to avoid any spilling.

3. Results

A total of 878 toothfish was tagged during the research cruise with a calculated total weight of 9677 kg, corresponding to 23% of the total catch in terms of both number and weight. Of these, 325 toothfish were tagged in the North (37%), 241 in the East (27%) and 312 in the South (36%) (Table 2). The number of tagged toothfish varied per day between 34 and 167, with an average of 73 ± 35 individuals. We tagged 355 toothfish with both small and large external spaghetti Floy® dart tags (FT-2-94 and FT-1-94, respectively) and 523 with only large external spaghetti Floy® dart tag (FT-1-94).

Toothfish tagging was prioritised during the first daily station, with the entire line monitored to ensure we reach the target number of 50-70 tagged toothfish daily (Table 2). The second station was used to reach the target number if not achieved during the first station. The percentage of the line monitored was thus lower for the second, compared to the first daily lines (Table 2). On station 1572, the low percentage of line monitored and fish tagged was due to a considerable number of toothfish caught and tagged at the beginning of the line (Table 2). On station 1578, we had to stop tagging due to the presence of sperm whales near the vessel, resulting in a low percentage of both the line monitored and fish tagged (Table 2).

Table 2. Tagging information (percentage of the line monitored by the scientist to tag toothfish and the percentage of tagged toothfish) and characteristics of the tagged toothfish (total length mean and standard deviation and, total length range) by station. The percentage of the line monitored by the scientist was calculated in term of time spend to tag and wait for suitable toothfish to tag. The percentage of tagged fish was calculated as the number of toothfish tagged according to the number of toothfish caught on the portion of the line monitored by the scientist. Stations are sorted by area and haul date.

Area	Date	Station	Percentage of line monitored	Percentage of tagged fish	Number of tagged fish	Total length (mean ± sd)	Total length range
North	21/10/2023	1566	100	48	51	98 ± 17	64 - 143
		1567	33	49	22	101 ± 14	81 - 124
	22/10/2023	1568	100	32	73	101 ± 17	70 - 141
		1569	35	28	24	112 ± 13	89 - 150
	23/10/2023	1570	100	28	55	108 ± 17	76 - 158
		1571	34	27	18	105 ± 18	75 - 141
	24/10/2023	1572	67	39	82	102 ± 15	63 - 140
East	20/10/2023	1564	100	47	37	110 ± 19	62 - 140
		1565	43	49	29	99 ± 16	67 - 125
	25/10/2023	1573	100	42	36	90 ± 16	61 - 139
	26/10/2023	1574	100	39	45	97 ± 18	67 - 141
		1575	72	38	33	107 ± 20	63 - 149
	27/10/2023	1576	100	33	40	98 ± 20	63 - 150
		1577	16	23	21	105 ± 18	72 - 149
South	28/10/2023	1578	34	22	43	92 ± 7	76 - 111
	29/10/2023	1579	100	38	42	94 ± 19	52 - 136
		1580	100	27	26	110 ± 16	78 - 140
	31/10/2023	1581	100	19	34	111 ± 22	72 - 170
	01/11/2023	1582	100	27	102	95 ± 12	65 - 124
		1583	68	29	65	101 ± 13	73 - 133

The length of tagged toothfish ranged from 52 to 170 cm (mean TL ± sd: 101 ± 17.1 cm) (Table 2 and Figure 3.a) and, the length of sampled fish (i.e. sampled for length-frequency and otoliths) ranged from 57 to 184 cm (mean TL ± sd: 101 ± 21.1 cm) (Figure 3.b).

There was no significant difference in length distribution between the tagged and sampled toothfish (Anova, p-value = 0.7709; Figure 3), and between the three sub-areas for sampled toothfish (Anova, p-value = 0.6379; Figure 3). However, the length distribution of tagged toothfish differed significantly between sub-areas (Anova, p-value = 0.004376 **); toothfish tagged in the South were significantly smaller than in the North (mean TL ± sd_{South}: 98.6 ± 15.8 cm, mean TL ± sd_{North}: 103 ± 16.2 cm; Figure 3).

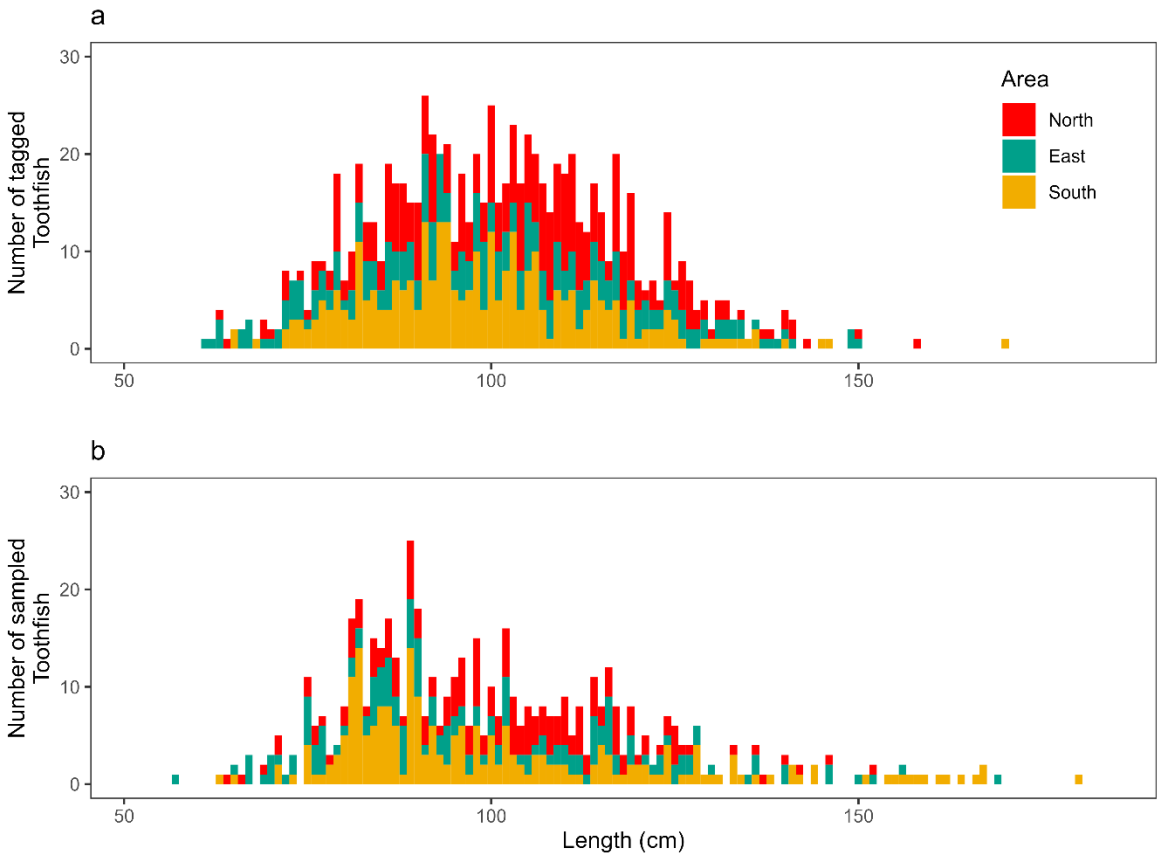

Figure 3. Cumulative length-frequency of (a) tagged and (b) sampled toothfish during the research cruise ZDLK3-10-2023 partitioned by sub-area (North, East and South, Figure 1). Sampled toothfish comprised random and sub-sample toothfish collected for length-frequency and otoliths.

A total of 107 gonads (62 female and 45 male gonads) were sampled during the ZDLK3-10-2023 research cruise. Among them, 31 were sampled in the North, 19 in the East, and 57 in the South, including 80 randomly sampled and 27 non-randomly sampled (Figure 4). The length of toothfish sampled for gonads ranged from 71 to 184 cm (mean TL ± sd: 119 ± 26.5 cm) (Figure 4). The length distribution of toothfish sampled for gonads was significantly different according to sex (Anova, p-value = 0.0219 *) and sampling type (Anova, p-value = 0.0158 *; Figure 4.b) but not according to sub-area (Anova, p-value = 0.8881; Figure 4.a). Females sampled for gonads were significantly larger than males (mean TL ± sd_{Female}: 124 ± 30.4 cm, mean TL ± sd_{Male}: 112 ± 18.1 cm).

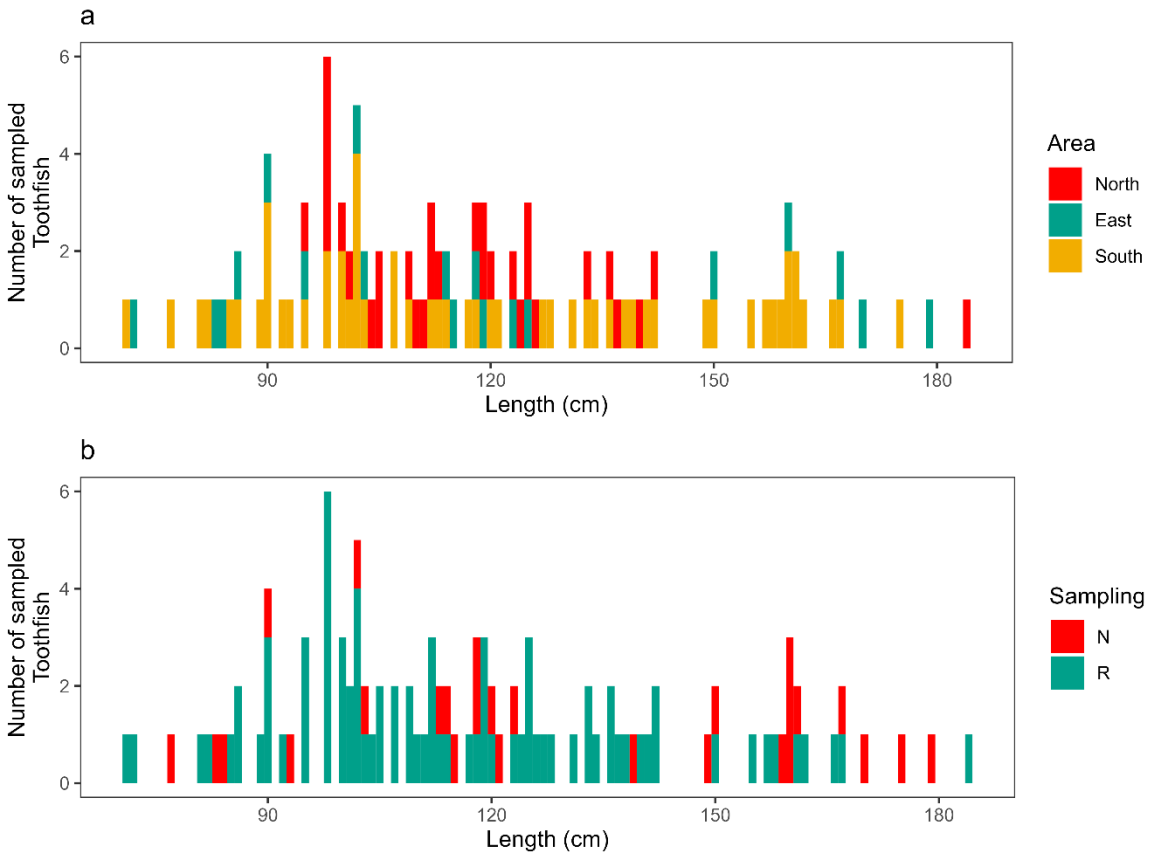

Figure 4. Cumulative length-frequency of toothfish sampled for gonads during the research cruise ZDLK3-10-2023, partitioned by (a) sub-area (North, East and South, Figure 1) and (b) type of sampling (N = Non-random and R = Random).

Maturity stages sampled were mainly stages 2 and 3 for both sexes (Table 3). One F4 was sampled at the station 1572 in the North (Figure 1) and three F7 were sampled in the East and South (stations 1576, 1580 and 1582, respectively; Figure 1). One M8 was sampled at station 1581 in the South (Figure 1).

Table 3. Number of toothfish sampled for gonads by sex and maturity stage (8-stage maturity scale developed by the FIFD; Brickle et al. 2006).

Maturity stage	Female	Male
2	28	13
3	30	31
4	1	-
7	3	-
8	-	1

4. Discussion

During the ZDLK3-10-2023 research cruise, we covered the entire toothfish fishing area within the Falkland Islands waters and we met the two aims of the research cruise by tagging 878 toothfish with external spaghetti Floy® dart tags and collecting the gonads of 107 toothfish.

Toothfish fishing area was divided into three sub-areas according to habitat characteristics: North, East and South and we planned to tag equal numbers of toothfish in each area. The number of tagged toothfish was equivalent in North and South areas (37% and 36%, respectively), and was slightly lower in the East (27%). The fishing effort and catches are a bit lower in the East (pers. com. Captain Garcia Portas F.) so we considered that a lower percentage of tagged toothfish in the East will not affect our understanding of fish movement and future stock assessment. However, the number of sub-areas, their delineations and the percentage of toothfish to be tagged per sub-area should be more formalised for the next tagging research cruise to coincide with the distribution of catch and/or effort. It may be interesting to consider the percentage of the catches by area, toothfish population distribution and the previous tagging location undergone by Scientific Fisheries Observers and previous tagging research cruises to set the research cruise tagging location.

The average soak time for this cruise was 705 ± 125 min, longer than the 550 ± 215 min at the ZDLK3-02-2018 research cruise (Farrugia and Keningale, 2018) and the 622 min at the ZDLK3-10-2022 research cruise (Nicholls and Raczynski, 2023). However, the comparison of the soak time between tagging cruises is unreliable due to inconsistencies in the calculation of the soak time between tagging research cruise reports (cf. Farrugia and Keningale, 2018; Skeljo and Pearman, 2021; Nicholls and Raczynski, 2023). At the meeting between the Toothfish scientist (Le Luherne E.), Stock assessment scientist (Skeljo F.) and Observer coordinator (Trevizan T.) it was agreed to calculate the soak time as the difference between the mid-set and mid-haul times. Previous findings suggested that the soak time did not impact the suitability of the condition of the toothfish (Skeljo and Pearman, 2021), however, we recommend to follow the advices of the captain avoiding long soak time in areas with scavengers as it seems to affect the suitability of toothfish for tagging.

We set 20 lines during the research cruise: 7 in the North, 7 in the East and, 6 in the South with the target of tagging 50-70 fish per day. While the daily number of suitable toothfish for tagging was highly variable, we reached the daily target tagging number of toothfish by tagging 73 toothfish on average. The length distribution of tagged toothfish was not significantly different from the length distribution of captured toothfish, in accordance with the target of the research cruise programme. The mean total length of tagged toothfish (mean ± sd: 101 ± 17.1 cm) was in the same order as Farrugia *et al*. (2018) and Nicholls and Raczynski (2023), 102.1 ± 13.7 cm and 98.4 ± 16.2 cm, respectively, and slightly higher

than Farrugia and Keningale (2018) and Skeljo and Pearman (2021) (94.5 ± 13.1 cm and 87.4 ± 13.7 cm, respectively).

These results are in accordance with our results highlighting that toothfish tagged in the South were significantly smaller than in the North. Farrugia and Keningale (2018) and Skeljo and Pearman (2021) covered mostly South and South-east sub-areas while Nicholls and Raczynski (2023) tagged toothfish in South and North sub-areas and Farrugia *et al*. (2018) covered the North-east sub-area.

This result was yet unexpected as Burdwood Bank, which is part of the South sub-area, is a known spawning area (Laptikhovsky *et al*. 2006) and should thus hold larger toothfish. Indeed, we sampled the largest individuals in the South (i.e. for length-frequency and otoliths). These results revealed that large toothfish were mainly unsuitable for tagging and seemed to be most frequently injured both during the catch process (e.g. with other hooks) and with the gaff to bring them onboard.

The outcomes for tagged fish seemed generally favourable, with toothfish reported swimming downwards a few seconds following release. We have not seen sperm whale actively feeding on the released tagged toothfish, but the predation of tagged toothfish by sperm whale may have occurred on their descent. We thus recommended to avoid and/or stop the tagging of toothfish in the reported presence of active predators such as sperm whales (*Physeter macrocephalus*) or killer whales (*Orcinus orca*).

The number of gonads and the maturity stages sampled for the toothfish reproductive strategy project were in accordance with expectations, and gonads were added to the collection of samples of this ongoing project.

To conclude, the ZDLK3-10-2023 research cruise achieved the targets defined in the scientific proposal and we made several recommendations for the next tagging research cruise.

Recommendations for the next tagging research cruise

- Define the tagging target as toothfish numbers per day instead of per line.
- Define the tagging sub-areas according to the historical longline fishery effort distribution, and allocate the tagging effort to each sub-area according to toothfish catch in numbers (or catch proportion) in the previous year/s per sub-area.
- Standardize the soak time calculation.
- Calculate the percentage of tagged toothfish per line by number instead of weight.
- Report the percentage of each line monitored by the tagging scientist.
- Record the daily weather conditions (wave height and wind speed) to assess any influence of these factors on toothfish suitability for tagging.
- Use a common R script to improve the replicability of the tagging research cruise outputs.

- Improve the hygiene of the tagging instruments to improve the cicatrisation of tagged toothfish and avoid infection around the tag. All the recaptured toothfish during this survey have developed an infection around the tags which raised concern about the hygiene of the tagging protocol.
- Avoid tagging in bad weather conditions as the quality of the caught toothfish seems to be affected (e.g. more toothfish were entangled in the rope and/or scratched by the umbrella under bad weather conditions).
- Avoid and/or stop tagging in the presence of sperm whales (*Physeter macrocephalus*) or killer whales (*Orcinus orca*).

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