Accord sur la Conservation des Cétacés de la Mer Noire, de la Méditerranée et de la zone Atlantique adjacente

Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic Area

Monitoring and mitigation of cetaceans' bycatch in Bulgarian waters

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1. CONTEXT OF THE PROJECT

There are three endemic subspecies of cetaceans inhabiting the Black Sea - Black Sea harbour porpoise *(Phocoena phocoena relicta* Abel, 1905), Black Sea bottlenose dolphin (*Tursiops truncatus ponticus* Barabash-Nikiforov, 1940) and Black Sea common dolphin (*Delphinus delphis ponticus* Barabash-Nikiforov, 1935). Commercial hunting of cetaceans in the Black Sea was intensive until 1966 when a ban was adopted by USSR, Bulgaria, and Romania, but it continued in Turkish waters until 1983. There are no complete and precise records of harvested numbers during that period but an estimated 4-5 million were taken in the 20th century (Birkun et al., 1992). Nowadays, all three Black Sea cetacean subspecies are protected by national and international legislation. Black Sea cetaceans face a number of threats such as pollution, habitat degradation, prey depletion, disturbance and especially incidental catch in fishing gears (Birkun, 2002). Bycatch (incidental catch) of small cetaceans is a major problem in a number of gillnet fisheries around the world (Read et al., 2006; Reeves et al., 2013). In Europe, cetacean bycatch is subject to the Habitats Directive 92/43/EEC, the Common Fisheries Policy (CFP), the Marine Strategy Framework Directive (MSFD) 2008/56/EC and the two regional agreements adopted under the auspices of the 1979 Convention for the Conservation of Migratory Species of Wild Animals (the “Bonn Convention”): Agreement on the Conservation of the Cetaceans of the Black Sea, Mediterranean and Contiguous Atlantic area (ACCOBAMS) and Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas (ASCOBANS). The EU Habitats Directive establishes that assessment of conservation status of species should be based on the information on status and trends of species populations and on the information on main pressures and threats. The EC Council Directive 56/2008 (Marine Strategy Framework Directive, MSFD) was adopted in 2008 and aims to achieve “Good Environmental Status (GES)” for the marine waters within the EU by 2020. Cetaceans are covered by descriptors: D1 Biodiversity, D4 Food webs, D8 Contaminants, D10 Marine litter, and D11 Underwater noise. Bycatch mortality, in relation to population status is one of the criteria assessed under descriptor D1. At national level in Bulgaria, no environmental targets and threshold values have been set for criterion D1C1 Bycatch due to lack of information on the values of bycatch by species and by fishery. Report on bycatch pilot for the Black Sea developed within CeNoBS project proposes to use 1.7% of population abundance accepted by ASCOBANS as threshold value for that criterion. That accounts for 1 598 to 4 386 porpoises annually and far exceeds estimated bycatch levels for the Black Sea of 11 826 to 16 200 porpoises (CeNoBS, 2021).

The Black Sea turbot gillnet fishery is considered one of the most important threats for small cetaceans due to bycatch (Birkun, 2002). The Black Sea turbot (*Scophthalmus maximus* Linnaeus, 1758) is the most valuable commercial fish species in the Black Sea. The EU regulates fishing activities of its Member States through the Common Fisheries Policy (CFP) and turbot fishery is managed through the annual establishment of EU quotas since 2008. Increase of quota from 114 to 150 t was made between 2019 and 2020, 50% share being divided between Bulgaria and Romania. Recommendation GFCM/37/2012/2 stipulated that turbot in the Black Sea (GSA29) should be fished exclusively by using bottom-set gillnets with a minimum stretched mesh size of 400 mm (200 x 200 mm). In Bulgaria, fishermen apply for a license to fish turbot each year and must comply with certain requirements – e.g., have no prior penalties for IUU fishing, use an automatic identification system (AIS) transponder, and also a Vessel Monitoring System (VMS). In 2020, a total of 124 fishing vessels were approved and granted licenses for turbot fishing in Bulgaria while following year that number increased to 126.

A seasonal ban on turbot fishing is usually in effect during the spawning period from mid-April to mid-June. In Bulgaria, the turbot fishing season is mainly in the spring (March-April) before the ban is enforced. Some fishermen also fish in the summer after the end of the ban. In autumn, turbot is rarely targeted because during that season migrating species are more abundant and preferred – bonito, horse mackerel, and bluefish.

Of all three small cetaceans inhabiting the Black Sea, harbour porpoise (*P. p. relicta*) is the most heavily and negatively affected by bycatch (Turkey – Tonay and Özturk, 2003; Gönener and Bilgin, 2009; Ukraine – Birkun Jr. et al., 2009; Bulgaria – Mihaylov, 2010). All of these studies report the largest share of bycatch to be of Black Sea harbour porpoise – 90 to 98%. Sustainable levels of bycatch for harbour porpoise have been calculated for the Western Black Sea based on an abundance estimation derived from a combined aerial and vessel distance-sampling survey in July 2013 (Birkun et al., 2014). Applying different approaches for defining sustainable bycatch rates (Potential Biological Removal; 1% and 2% limit by International Whaling Commission and 1.7% limit by ASCOBANS) indicate varying numbers of between 247 to 589 individuals (Birkun Jr. et al., 2014).

Pingers have been developed in the USA in the 1990s and EU Council Regulation No 812/2004 laid down measures concerning incidental catches of cetaceans in fisheries requiring member states to report bycatch levels and use pingers as a mitigation measure to reduce incidental catches of some small cetacean species and populations. Black Sea fisheries, however, were not covered under the Regulation, meaning Bulgaria and Romania, as EU members, were not enforced to implement it. Technical specifications described in both US and EU regulations had similar requirements for the pingers: an instrument, which when immersed in water, broadcasts a 10 kHz or 20-160 kHz sound at 130-150 dB re 1 μPa at 1m, lasting 300 ms, and repeating every 4 s. Two trials in Turkish waters of the Black Sea using different models of pingers have shown completely different results (Gönener and Bilgin, 2009; Bilgin and Köse, 2018). In Bulgaria, trials with pingers (10 kHz Future Oceans) showed reductions in pound nets (dalyan) depredation by porpoises and bottlenose dolphins (Zaharieva et al., 2016) and 100% reduction in bycatch by bottom-set gillnets in a turbot fishery in 2017-2019 (Zaharieva et al., 2019).

The current study is continuation of 2019 pilot aimed at estimating cetacean bycatch rates in the Bulgarian Black Sea turbot fishery and to assess the effect of pingers for reduction of bycatch. This involved on-board monitoring of fishing activities aboard several vessels specialized in turbot fishing. Three models of pingers are evaluated as a potential mitigation measure for cetacean bycatch, especially of the endangered Black Sea harbour porpoise.

2. ACTIVITIES CARRIED OUT DURING THE REPORTING PERIOD

2.1. ONBOARD MONITORING OF CETACEAN BYCATCH AND EFFECT OF PINGERS' USE

The trials planned to be conducted during the project were based on the previous experience and established good cooperation with 5 fishing vessels' shipmasters. Those were operating from Balchik port in the Northern Bulgaria while in Southern Bulgaria from Tsarevo and Primorsko. One more fisherman operating from Nessebar offered to join the trials, providing an important addition for the Central sector of Bulgarian Black Sea waters. That fact required an increase of pingers to create reasonable active sample of operated nets. Accordingly, 31 additional pingers of the 70 KHz Future Oceans model were purchased.

Given the fact that the two models (10 and 70 KHz) of Future Oceans pingers used in the first trial in 2019 provided mixed results we have looked for other available models on the market. Market research revealed that the options available were quite limited. For instance, two pinger producers, namely Dukane and Aquamark, used in previous tests in the Black Sea have ceased production. Banana pingers by Fishtek Marine, UK were unsuccessfully used in the past by some Bulgarian fishermen and therefore were not used either. One device, presented at the ECS Conference 2014 in Malta, producing porpoise alert signals, developed in Germany by prof. Boris Culik and produced by F^3: Maritime Technology company, looked rather promising. Prof. Culik was contacted in December 2019 by the project's personnel. Since the price of these devices was considerably higher than Future Oceans pingers, which were those originally budgeted in the proposal, prof. Culik provided a set of 40 devices for free trial under the condition that costs for transportation were covered and if any device was lost or damaged it would be paid. Since the specific model using alerting signal of porpoises was based on the Baltic subspecies its effectiveness in the Black Sea was uncertain. Consequently, it was decided to use another model based on the highly successful but no longer produced Dukane NetMark - 10 KHz-PALs (Porpoise ALerting devices).

Distribution of pingers and length of operated gillnets by fishing vessels and regions are presented in table 1 below:

Table 1: Fishing vessels participating in the project

Region	Vessel	Pingers	Control nets, m	Active nets, m	Total nets, m	Year
North	Vessel 1	120/80	17 080	17 080	34 160	2020
North	Vessel 1	66	9240	22 680	31 920	2021
North	Vessel 2	45	12 300	4 500	16 800	2020
North	Vessel 2	35	0	3100	3100	2021
Central	Vessel 3	29	20 300	6 000	26 300	2020
South	Vessel 4	10	2 000	2 000	4 000	2020
South	Vessel 4	10	2 000	0	2 000	2021
South	Vessel 5	40	0	6 000	6 000	2020
South	Vessel 5	40	0	6 000	6 000	2021
South	Vessel 6	10	5 500	0	5 500	2020
Central	Vessel 7	29	11 500	2 500	14 000	2021
Total			**79 920**	**69 860**	**149 780**	

Vessels in red have not operated.

All pingers described above were handed to fishermen who agreed to take part in the trial. Vessel 1 being the largest and operating the longest sets of nets was

Figure 1: Setting of gillnets equipped with PAL 10 KHz

designated to test new model of pinger – PAL 10 KHz. In spring 2020, we started with provided set of 40 PALs. For the summer 2020 trials, that number was increased to 80

PALs thanks to additional 40 pcs provided by prof. Culik. During summer 2020 trials three PALs were lost due to the net being ripped by a trawler, while other seven were damaged by flooding during operation. As a consequence, in 2021, there were 66 PALs available for trials, which took place exclusively onboard vessel 1.

Vessels 3 and 7 operated from Nessebar in Central sector but each of these joined only for one year of the two-year period of the study. In 2021, vessel 7 had a breakdown during spring haul of nets and only small portion of set nets was observed but since none of the pingers were deployed trial was not valid anyway. In the summer one of the five strings was equipped with Future Oceans 10 KHz pingers.

Vessels 4 and 5 operating from port of Primorsko in the South have cancelled operation in 2020 due to COVID-19 pandemic and enforced emergency situation in Bulgaria. Being a small town and these fishermen being part-time and seasonal they opted for safety rather being on a risk. Additional factor for non-fishing in spring was unfavourable weather conditions during normal period for setting of nets: second part of March and beginning of April. Continuous strong Northeastern winds for more than a week formed heavy swell. Since these two vessels were the smallest in size – approx. 8 m length these conditions were significant obstacle for their operation. These weather conditions were further complemented by formation of local currents in depth preventing normal operation of bottom set gillnets. In this way Southern sector remained with only one operating vessel from Tsarevo – vessel 6. Though, that vessel has not used the provided pingers but given the fact that in 2019 shipmaster had not complied to recommended spacing of pingers it was not a big issue and was used as control. In 2021, vessel 4 operated one set of nets only but no pingers were fixed to it.

Maps showing overview of all operated active and control nets participating in the project in spring 2020 can be seen as figure 2; in summer 2020 as figure 3; in spring 2021 as figure 4 and in summer 2021 as figure 5:

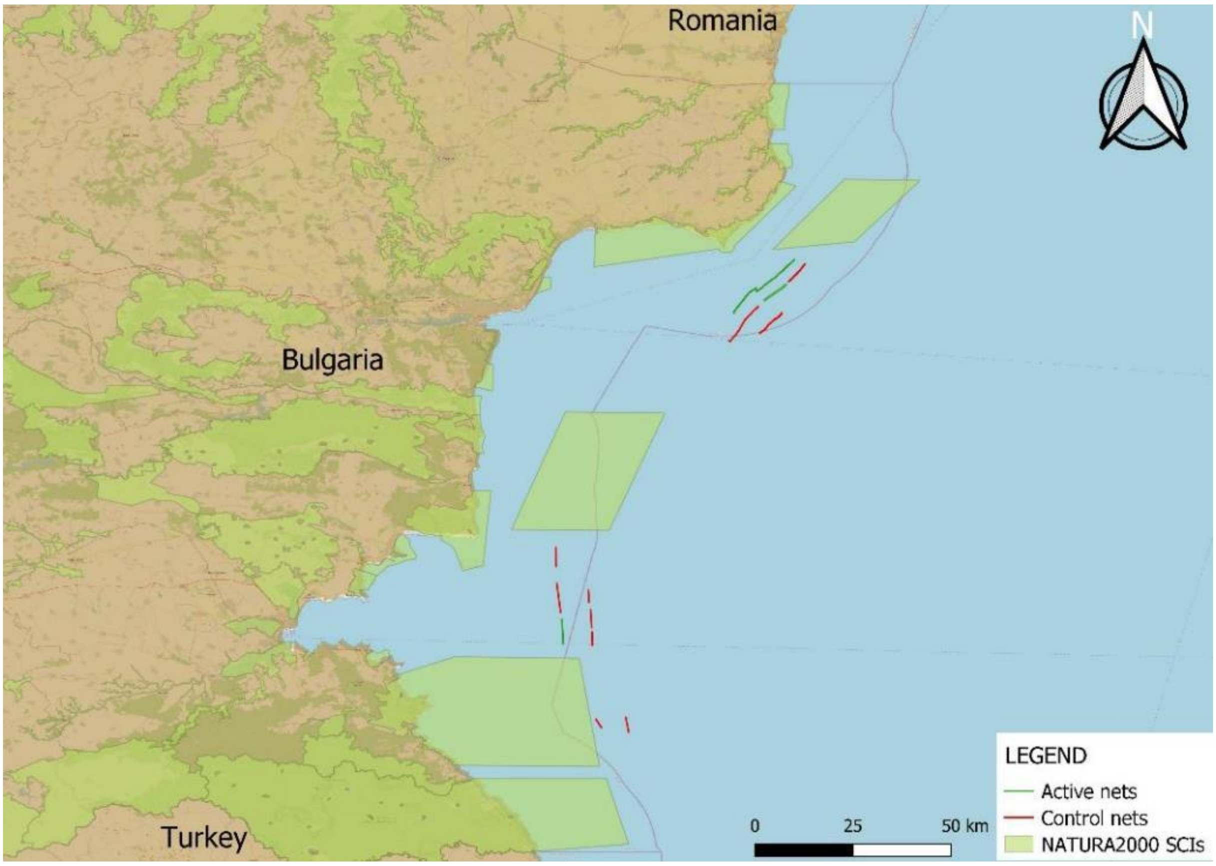

Figure 2: Map with position of nets set by vessels participating in the trial in spring 2020

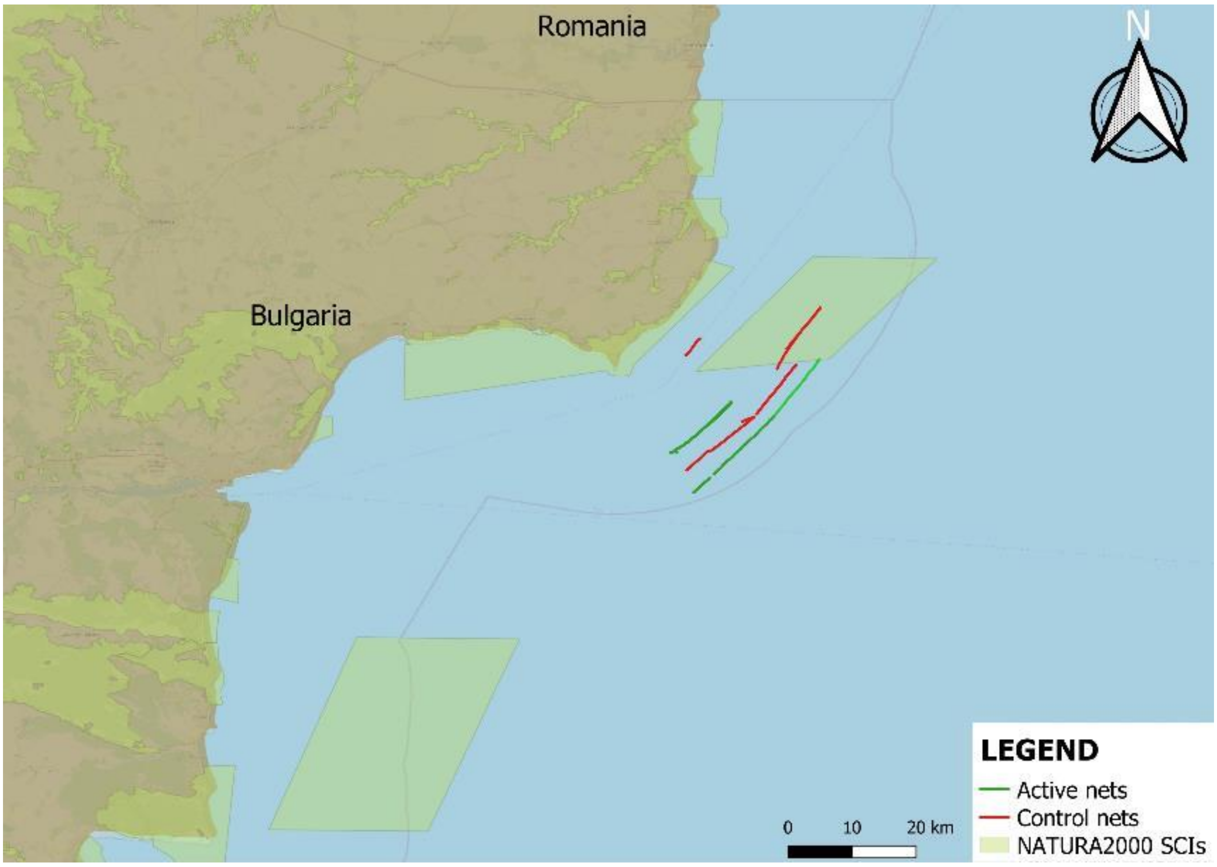

Figure 3: Map with position of nets set by vessels participating in the trial in summer 2020

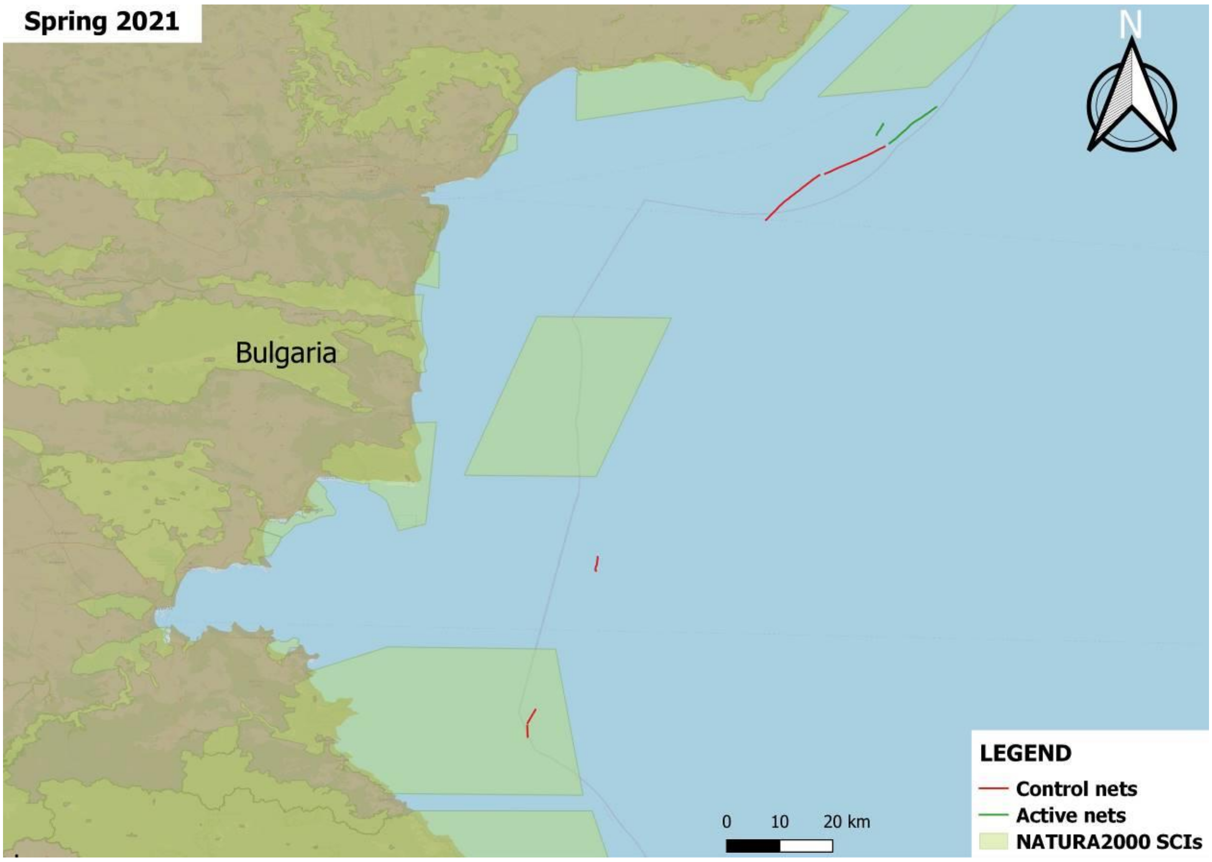

Figure 4: Map with position of nets set by vessels participating in the trial in spring 2021

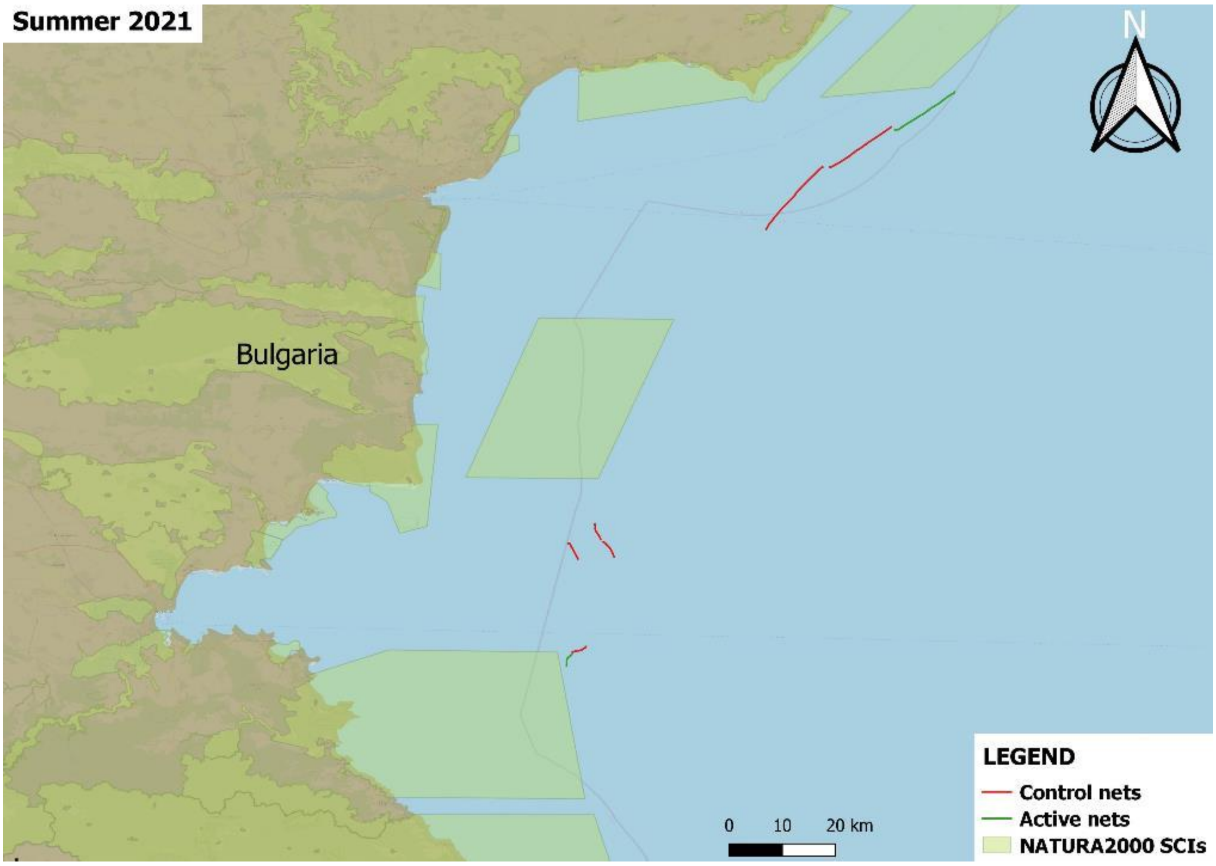

Figure 5: Map with position of nets set by vessels participating in the trial in summer 2021

Summarized data on trials and effort by vessel, date and year including configuration and spacing for each set, soaking time, length, depth type of sampling and existence of bycatch are presented in table 2 below:

Table 2: Summarized data on configuration and effort for each trial

Boat	Date	Pingers/ spacing	Soak time, days	Length, m	Effort (km^2*days)	Depth m	Sampling	By-catch (Y/N)
1	10.4.2020	no	21	5600	0,35	74	observer	Y
1	10.4.2020	PAL-140m	21	5600	0,35	74	observer	N
1	12.4.2020	FO-10kHz/140m	22	11480	0,76	65	observer	Y
1	13.4.2020	no	24	11480	0,83	76	observer	Y
2	12.4.2020	FO-10kHz/100m	14	4500	0,16	65	observer	N
2	12.4.2020	no	14	4300	0,16	65	observer	N
2	12.4.2020	no	14	8000	0,29	83	observer	Y
6	13.4.2020	no	17	4200	0,18	65	self	N
6	13.4.2020	no	17	6100	0,26	65	self	N
6	13.4.2020	FO-70kHZ/200m	17	6000	0,26	65	self	N
6	13.4.2020	no	17	3000	0,13	75	self	N
6	13.4.2020	no	17	4000	0,17	75	self	N
6	13.4.2020	no	17	3000	0,13	75	self	Y
3	10.4.2020	no	31	2300	0,21	80	self	Y
3	12.4.2020	no	15	3200	0,14	82	observer	N
1	28.6.2020	PAL-140m	12	11200	0,40	80	observer	Y
1	2.8.2020	PAL-140m	10	10640	0,32	70	observer	Y
1	4.7.2020	no	14	10360	0,44	68	observer	Y
1	16.7.2020	PAL-140m	12	11200	0,40	77	observer	Y
1	23.7.2020	no	11	11000	0,36	76	observer	Y
1	29.7.2020	no	13	11200	0,44	75	observer	Y
2	28.6.2020	FO-10kHz/100m	12	3100	0,09	81	observer	N
2	14.10.2020	no	7	3100	0,05	45	observer	N
1	10.4.2021	no	14	10080	0,42	80	observer	Y
1	13.4.2021	no	13	11760	0,46	80	observer	Y
1	11.4.2021	PAL-140m	15	9240	0,42	78	observer	N
1	11.4.2021	no	15	840	0,04	78	observer	Y
2	11.4.2021	FO-10kHz/100m	15	2500	0,09	82	observer	N
7	12.4.2021	no	16	2700	0,11	86	observer	N
4	13.4.2021	no	15	2000	0,08	70	self	Y
1	2.7.2021	no	12	10920	0,39	80	observer	Y
1	3.7.2021	PAL-140m	13	9240	0,36	80	observer	N
1	3.7.2021	no	13	1680	0,07	80	observer	Y
1	4.7.2021	no	14	11760	0,49	80	observer	Y
2	4.7.2021	FO-10kHz/100m	14	2600	0,09	80	observer	N

7	15.7.2021	no	25	3000	0,15	81	observer	N
7	15.7.2021	no	25	3300	0,17	87	observer	Y
7	15.7.2021	no	25	3000	0,15	77	observer	N
7	15.7.2021	no	26	2200	0,11	83	observer	N
7	15.7.2021	FO-10kHz/100m	26	2500	0,13	81	observer	N

Configuration of active and control strings of nets by vessels and by year and season are described in detail below.

<u>Vessel 1</u>

Spring 2020

Normally that vessel operated 3 sets, each being approx. 11 200 m but for the purpose of testing effect of PALs and given limited number of 40 pcs available, one of the sets was separated to two shorter ones.

Set 1: 5 600 m control without pingers.

Set 2: 5 600 m active fitted with 40 PALs spaced at 140 m. Soaking time for these two sets was 21 days while distance between them was about 800 m.

Set 3: 11 480 m active fitted with 80 pingers Future Oceans 10 KHz spaced at 140 m. Soaking time of that set was 22 days.

Set 4: 11 480 m control without pingers. Soaking time of that set was 24 days.

Summer 2020

After additional PALs were provided by producer and total number reached 80, fisherman got back to normal commercial practice by using longer strings. Rotation through the summer allowed to have three active and three control strings.

Set 1: 11 200 m active fitted with 80 PALs spaced at 140 m. Soaking time was 12 days.

Set 2: 11 200 m control net with soaking time 14 days. 840 m of nets were missing during hauling being broken by a trawler.

Set 3: 11 200 m active fitted with 80 PALs spaced at 140 m. Soaking time was 12 days.

Set 4: 11 200 m control without pingers. Soaking time of that set was 11 days. 200 m were missing during hauling being broken by a trawler.

Set 5: 11 200 m control without pingers. Soaking time of that set was 13 days.

Set 6: 11 200 m active fitted with 80 PALs spaced at 140 m. Soaking time was 10 days. 560 m of nets were missing during hauling being broken by a trawler including 3 PALs.

Spring 2021

Set 1: 10 080 m control without pingers. Soaking time of that set was 14 days.

Set 2: 10 080 m of which 9 240 m active fitted with 66 PALs and 840 control. Soaking time of that set was 15 days.

Set 3: 11 760 m control without pingers. Soaking time of that set was 13 days.

Summer 2021

Set 1: 10 920 m control without pingers. Soaking time of that set was 12 days.

Set 2: 10 920 m of which 9 240 m active fitted with 66 PALs and 1680 m were control. Soaking time of that set was 13 days.

Set 3: 11 760 m control without pingers. Soaking time of that set was 14 days.

<u>Vessel 2:</u>

Spring 2020

Set 1: 8 800 m, 4 500 m of which were active fitted with 45 pingers Future Oceans 10 KHz

Set 2: 8 000 m control without pingers.

Both sets have been soaked for 14 days.

Summer 2020

Set 1: 3 100 m fitted with 31 pingers Future Oceans 10 KHz. Soaking time 12 days.

Autumn 2020

Set 1: 3 100 m control net with soaking time of 7 days.

In 2021 vessel 2 has stopped operation but its nets have been used by vessel 1 on basis of agreement between two fishermen.

Spring 2021

Set 1: 2 500 m fitted with 25 pingers Future Oceans 10 KHz. Soaking time 15 days.

Summer 2021

Set 1: 2 600 m fitted with 26 pingers Future Oceans 10 KHz. Soaking time 14 days.

Vessel 3

Spring 2020

Set 1: 4 200 m control without pingers.

Set 2: 6 100 m control without pingers.

Set 3: 6 000 m active fitted with 29 pingers Future Oceans spaced at 200 m.

Set 4: 3 000 m control without pingers.

Set 5: 4 000 m control without pingers.

Set 6: 3 000 m control without pingers.

Soaking time for all 6 sets was 17 days.

Vessel 4

Spring 2021

Set 1: 1 000 m control without pingers. Soaking time 15 days.

Set 2: 1 000 m control without pingers. Soaking time 15 days.

Vessel 6

Spring 2020

Set 1: 2 300 m control without pingers. Soaking time 31 days.

Set 2: 3 200 m control without pingers. Soaking time 15 days.

Vessel 7

Spring 2021

Set 1: 2 700 m control without pingers. Soaking time 16 days.

Summer 2021

Set 1: 3 000 m without pingers. Soaking time 25 days.

Set 2: 3 300 m without pingers. Soaking time 25 days.

Set 3: 3 000 m without pingers. Soaking time 25 days.

Set 4: 2 200 m without pingers. Soaking time 26 days.

Set 5: 2 500 m fitted with 25 pingers Future Oceans 10kHz. Soaking time 26 days.

RESULTS

External status of observed carcasses entangled in the fishing gear during the study are classified according codes described in “Best practice on cetacean post mortem investigation and tissue sampling” by L. IJsseldijk, A. Brownlow and S. Mazzariol (2019), adopted by ACCOBAMS during 7th Meeting of Parties. Summarized data showing all trials by date, vessel, bycatch as individuals, standardized effort (square kilometer of nets by days) and bycatch rate of individuals per effort, depth and type of sampling (by observer or self-sampling by fisherman) is shown in table 3 below:

Table 3: Summarized monitored effort and recorded bycatch by individuals, species and standardized as ind./effort

Boat	Date	Pinger model /spacing	By-catch (ind.)	Effort (km^2 * days)	Bycatch (ind / effort)	Type of sampling	Species		
							Dd	Pp	Tt
1	10.4.2020	no	2	0,35	5,67	observer	1	1	
1	10.4.2020	PAL-140m	0	0,35	0,00	observer			
1	12.4.2020	FO-10kHz/140m	2	0,76	2,64	observer		2	
1	13.4.2020	no	2	0,83	2,42	observer		2	
2	12.4.2020	FO-10kHz/100m	0	0,16	0,00	observer			
2	12.4.2020	no	0	0,16	0,00	observer			
2	12.4.2020	no	1	0,29	3,43	observer	1		
6	13.4.2020	no	0	0,18	0,00	Self-report			
6	13.4.2020	no	0	0,26	0,00	Self-report			
6	13.4.2020	FO-70kHZ/200m	0	0,26	0,00	Self-report			
6	13.4.2020	no	0	0,13	0,00	Self-report			
6	13.4.2020	no	0	0,17	0,00	Self-report			
6	13.4.2020	no	1	0,13	7,84	Self-report			1
3	10.4.2020	no	1	0,21	4,68	Self-report		1	
3	12.4.2020	no	0	0,14	0,00	observer			
1	28.6.2020	PAL-140m	6	0,40	14,88	observer		6	
1	2.8.2020	PAL-140m	1	0,32	3,13	observer	1		
1	4.7.2020	no	14	0,44	32,18	observer		14	
1	16.7.2020	PAL-140m	4	0,40	9,92	observer		4	
1	23.7.2020	no	10	0,36	27,55	observer		10	
1	29.7.2020	no	3	0,44	6,87	observer		3	
2	28.6.2020	FO-10kHz/100m	0	0,09	0,00	observer			
2	14.10.2020	no	0	0,05	0,00	observer			
1	10.4.2021	no	4	0,42	9,45	observer		3	1
1	13.4.2021	no	3	0,46	6,54	observer		2	1
1	11.4.2021	PAL-140m	0	0,42	0,00	observer			
1	11.4.2021	no	1	0,04	26,46	observer		1	

2	11.4.2021	FO-10kHz/100m	0	0,09	0,00	observer			
7	12.4.2021	no	0	0,11	0,00	observer			
4	13.4.2021	no	2	0,08	26,67	self		2	
1	2.7.2021	no	7	0,39	17,81	observer		7	
1	3.7.2021	PAL-140m	0	0,36	0,00	observer			
1	3.7.2021	no	3	0,07	45,79	observer		3	
1	4.7.2021	no	10	0,49	20,25	observer		10	
2	4.7.2021	FO-10kHz/100m	0	0,09	0,00	observer			
7	15.7.2021	no	0	0,15	0,00	observer			
7	15.7.2021	no	1	0,17	6,06	observer		1	
7	15.7.2021	no	0	0,15	0,00	observer			
7	15.7.2021	no	0	0,11	0,00	observer			
7	15.7.2021	FO-10kHz/100m	0	0,13	0,00	observer			
		TOTAL					**3**	**72**	**3**

Spring 2020

Vessel 1:

Set 1 (control without pingers) was hauled on 10 April. During haul of that set, two cetaceans have been found – a highly decomposed (code 4) juvenile female of harbour porpoise (length = 73 cm) and a female common dolphin (code 3) starting to decompose with the skin beginning to peel (length = 159 cm). Two water birds were found – one Coot (*Fulica atra*) and one Moorhen (*Gallinula chloropus*). Catch was 15 kg of turbot and 2 kg of Thornback ray (*Raja clavata*). Position of nets during haul suggested these were not vertical being wound by sea current and that causing the main reason for low catch. Since decomposition of cetaceans has started for the common dolphin and being quite advanced for the juvenile porpoise it is most likely these two cetaceans have been bycaught in the first days of gillnets setting. Two water birds were also decomposed and since depth was 74 m these most probably were not bycaught but brought into the nets by current. In addition, Coot and Moorhen are typical freshwater inhabitants even though former frequents sea in winter but both species do not dive to such great depth.

Figure 6: Juvenile female Black Sea harbour porpoise from set 1

Figure 7 and 8: Female Black Sea common dolphin found in set 1

Set 2 (active fitted with 40 PALs spaced at 140 m) was hauled on 10 April: No entangled cetaceans have been found. Catch of turbot was 10 kg and thornback ray – 3 kg.

Set 3 (active fitted with 80 pingers Future Oceans 10 KHz spaced at 140 m) was hauled on 12 April. During haul it has turned out that most of the pingers were not operating due to exhausted batteries. With that finding in mind recording of operating and non-operating pingers was made with final number showing 21 operating and 59 inactive pingers. Since all pingers have been checked before attachment to nets and those whose batteries were exhausted have been replaced with brand new, it became clear that the LED indicators are not showing properly the battery level. Former studies have revealed that non-compliance to spacing between pingers or non-operating devices cause actually a higher bycatch with hypotheses being that these gaps are identified as safe passages so we paid special attention to that case. In total, 5 cetaceans have been found entangled in that set: 4 were harbor porpoises with last one being just a rear piece of body with tail fluke most probably also being a porpoise entangled post-mortem. It is interesting fact that on the same day during haul of set 2 of vessel 2 front part of decomposed porpoise was recorded but judging by position of two sets (between them were sets 1 and 2 of vessel 1) it is not likely it was the same animal.

Figure 9: Entangled male neonate harbour porpoise in set 3

Figure 10: Entangled porpoise neonate of unidentified sex

First harbour porpoise dropped during the haul and could not be recorded in detail or photographed but it was definitely adult and most probably bycaught judging by its

Figure 11: Bycaught lactating female porpoise

relatively preserved exterior (code 2). It was in a section and position between two non-active pingers so could be attributed to that fact. Second and third were decomposed neonates (code 4) – male, 41 cm (fig. 9) and unidentified sex, 37 cm (fig. 10) – that were also in section with inactive pingers. Fourth was clearly bycaught (code 2) lactating female, 128 cm (fig. 11) that was positioned between two operating pingers. Results from that set once again were mixed with one bycaught porpoise being in operating pingered section and second being in non-operating pingered section. That outcome cannot confirm reduction of bycatch through use of Future Oceans 10 KHz pingers in multifilament nets. That set was at depth of 65 m and catch of turbot with 12 kg and thornback ray with 5 kg was even lower than first two sets that had almost identical total length.

Set 4 (control without pingers) was the last hauled on 13 April being fixed at depth of 76 m. Once again catch of turbot and thornback ray was very low: 22 and 6 kg respectively suggesting unproper operating of bottom set gillnets due to strong currents. Two cetaceans were found during the haul – both being Black Sea harbour porpoises (code 2) and considered as bycatch. First one was a sad finding of female in advanced pregnancy with length of 116 cm and girth of 85 cm. During performed necropsy, foetus was identified as female with a length of 52 cm. Second porpoise

Figure 12 and 13: Pregnant female harbour porpoise and female foetus dropped during the haul and could not be described properly but was in relatively fresh state suggesting bycatch and being identified as adult.

Vessel 2:

Set 1 (8 800 m, 4 500 m of which were active fitted with 45 pingers Future Oceans 10 KHz): No entangled cetaceans were found during haul of that monofilament set that was at 65 m depth. Catch of turbot and thornback ray was relatively low – approx. 30 kg of former and 5 kg of latter.

Set 2 (control without pingers): That set was situated at the largest depth – 83 m and had the largest catch of 190 kg turbot and almost 800 kg of thornback ray. It was multifilament and two cetaceans were detected during the haul – one Black Sea common dolphin (code 3) and one Black Sea harbour porpoise (code 4) with missing rear part. Both of these though dropped during the haul and could not be recorded in detail and we consider the common dolphin to be bycaught while the incomplete carcass of porpoise to be entangled post-mortem. Short time of soaking for that set – 14 days and largest depth – make decomposition less likely given low temperature but on the other hand high fish catch ensures gillnets were operating properly. That was second case during that campaign (out of three for the entire study) of finding common dolphin entangled in bottom set gillnets – species that was considered not affected by that fishing gear in the Black Sea. Those cases should be noted with care and used as reference in future studies.

Vessel 3

That vessel had no independent observers due to smaller size, coincidence of haul with other two vessels and very long time spent offshore – 19 hours. All these reasons prevented full record in detail of catch and bycatch. One of the fishermen (who was the main contact for involvement of the vessel into the trial) has volunteered to record data on bycatch. All nets were monofilament.

Sets 1 and 2 (control) **and 3** (active fitted with 29 pingers Future Oceans spaced at 200 m): no bycatch was recorded in all these three sets that were at 65 m depth.

Sets 4, 5 and 6 (all being control without pingers): those sets were at 75 m depth and while in first two no bycatch was recorded in the last one was reported bycatch of one bottlenose dolphin that was dropped during haul. Approximate size was 180 - 200 cm and sex was not identified. Total catch for all 6 sets was 120 kg of turbot and 20 kg of thornback ray.

Vessel 6

Set 1 (control without pingers): That set was at depth of 80 m and one bycaught harbour porpoise was recorded that dropped during the haul. Total catch was 18 kg of turbot and 3 kg of thornback ray.

Set 2 (control without pingers): That set was at depth of 82 m and no bycatch of cetaceans was reported by the shipmaster who has committed to do a self-assessment. Total catch was 30 kg of turbot and 35 kg of thornback ray.

On several occasions bycaught cetaceans have been reported as dropped and following clarification should be made. Dropping from nets is a common case during haul of turbot nets and it happens when the animal is not entangled on upper or lower rope but only on twine that usually snaps under the weight when body is dragged from water. Smaller vessels usually cannot haul dead cetacean onboard but either untangle or cut the twine and release it before boarding.

Summer 2020

Vessel 1

Set 1 (active fitted with 80 PALs spaced at 140 m) was hauled on 28 June 2020. That set was at depth of 79-80 m. 6 porpoises (all were code 2) have been found as bycatch – 5 were female and 1 male. Sizes were 127 cm of male and 133-144 cm of females. Two of the bycaught porpoises have been eaten by spiny dogfish. Catch was 105 kg of turbot; 462 kg of thornback ray and 10 kg of spiny dogfish.

Figure 14: Porpoise bitten by spiny dogfish

Set 2 (control net without pingers) was hauled on 4 July 2020. 840 m of nets were missing during hauling being broken by a trawler and thus effectively observed nets were with length of 10 360 m. Fish catch was 70 kg of turbot and 155 kg of thornback ray. Bycatch was more than twice higher compared to active string hauled a week earlier and was 14 porpoises (all were code 2) – 8 females and 4 males with other 2 dropping during the haul preventing the sex identification. Length of males was 114-144 cm and of females 125-136 cm. Actually, in total 5 of the porpoises dropped during the haul but for three of these, sex was identified before sinking. Stomachs were collected of 8 animals for the microplastics study together with teeth for age determination.

Figure 15: Bycaught porpoises

Figure 16: Lesions on dorsal fin of male porpoise

Set 3 (active fitted with 80 PALs spaced at 140 m) was hauled on 16 July 2020. Bycatch was 4 porpoises (all were code 2) – 2 females (130 and 135 cm) while other 2 dropped during haul and sex was not identified. One of the females was lactating. One of the dropped porpoises was next to damaged PAL and that could have been the cause of that particular bycaught individual but that is only a hypothesis. Fish catch was 70 kg of turbot and 80 kg of thornback ray.

Figure 17: Female porpoise with clear net marks

Figure 18: Porpoise during haul of net

Set 4 (control without pingers) was hauled on 23 July 2020. That was the set with highest bycatch in 2020 – 10 porpoises (all were code 2). 8 were female (101-133 cm) and 1 male (127 cm) with remaining one being dropped during the haul. Two of the females were lactating. Fish catch was 60 kg of turbot and 80 kg of thornback ray. 200 m of the nets were missing being broken by a trawler.

Figure 19: Bycaught female Black Sea harbour porpoise

Set 5 (control without pingers) was hauled on 29 July 2020. Bycatch was 3 porpoises (all were code 2) – 2 females (121 and 150 cm) and 1 male (135 cm). It should be noted that both females were entangled close to each other. Fish catch was 40 kg of turbot and only 10 kg of thornback ray.

Figure 20: Two female porpoises that were entangled close to each other.

Set 6 (active fitted with 80 PALs spaced at 140 m) was hauled on 2 August 2020. 560 m of nets were missing during the haul being broken by a trawler including 3 PALs and thus actually 10 640 m of nets were observed. One bycaught common dolphin was recorded but it dropped during the haul. Fish catch was 40 kg of turbot and 20 kg of thornback ray.

Vessel 2

Set 1 (active equipped with 31 pingers Future Oceans 10 KHz) was hauled on 28 June 2020. No cetaceans were entangled in that monofilament string while fish catch was 45 kg of turbot and 15 kg of thornback ray.

Autumn

Vessel 2

Set 1 (control without pingers) was hauled on 14 October 2020. No bycatch was recorded but it was concluded that the net was robbed as fish catch was exclusively thornback ray – 150 kg and only one turbot. Many holes of the nets have been observed suggesting caught turbot was collected. The string was also broken at one end additionally suggesting manipulation. It is not likely that there were any bycaught cetaceans as obviously target of the robbers was only turbot and presumably, they were in a hurry.

Spring 2021

Vessel 1

Set 1 (control without pingers) was hauled on 10 April 2021. Entangled in the nets were 4 porpoises and 1 bottlenose dolphin. First porpoise was 57 cm long and in advanced stage of decomposition (code 4) not allowing sex identification and suggesting possible entanglement post-mortem. Two of the other porpoises (code 2) were females (130 and 132 cm) – both pregnant with male foetuses. Last porpoise (code 2) was male – 100 cm. Bottlenose dolphin (code 2) was also female (202 cm). Fish catch was rich: 390 kg of turbot, 720 kg of thornback ray and 26 kg of spiny dogfish.

Figure 21: Bycaught bottlenose dolphin

Figure 22: Pregnant porpoise

Set 2 (10 080 m of which 9 240 m active fitted with 66 PALs and 840 control without pingers) was hauled on 11 April 2021. In the active part of the set no bycatch was recorded but in the control piece one porpoise (code 2) was found. Unfortunately, it was again pregnant female (129 cm) and foetus once again was male.

Set 3 (control without pingers) was hauled on 13 April 2021. Recorded bycatch was of 2 porpoises and 1 bottlenose dolphin (all were code 2). All animals were female and one of the porpoises dropped during the haul.

Vessel 2

Set 1 (active fitted with 25 pingers Future Oceans 10 KHz at 100 m) was hauled on 11 April 2021. No bycatch was recorded.

Vessel 4

Set 1 and set 2 (both were monofilament 1 000 m each control without pingers) were hauled on 13 April 2021. That was one of the smaller boats that couldn’t accommodated observer but fisherman has reported himself bycatch of one porpoise per set – one being male and the other female but no size was measured. Fish catch was 130 kg of turbot and only 5 kg of thornback ray.

Vessel 7

Set 1 (2 700 m control without pingers) was hauled on 12 April 2021. Actual length was approx. 6 km but due to a breakdown haul was interrupted. No bycatch was observed during haul of that part of the set while catch was 20 kg of turbot and 40 kg of thornback ray.

Summer 2021

Vessel 1

Set 1 (control without pingers) was the first to be hauled on 2 July 2021. Seven porpoises were bycaught (all were code 2) – 4 females (122-128 cm) and 3 males (112-122 cm). Teeth were collected from three females and two males together with two and one stomach respectively. Fish catch was mainly thornback ray – 1020 kg and only 10 kg of turbot.

Figure 23: Maximum number of bycaught porpoises during a single haul in 2021 was seven

Set 2 (10 920 m of which 9 240 m active fitted with 66 PALs and 1680 m were control) was hauled on following day – 3 July 2021. No bycatch was recorded in the active part confirming excellent results of PALs in 2021 with zero bycatch. Control part further showed important role of PALs as 3 porpoises (all were code 2) were found there – two of these dropped during the haul but one was taken on board. It was lactating female of 115 cm. Teeth and stomach were collected. Fish catch was again almost entirely thornback ray – 960 kg and only 12 kg of turbot.

Set 3 (control without pingers) was hauled on 4 July 2021. That was the set with highest bycatch: 10 porpoises (code 2) – 3 males (117-124 cm) and 7 females (123-133 cm). All females were lactating. Stomachs and teeth were collected of two females and one male. Skin lesion was observed on one of females and sample was collected for histological examination. Turbot catch was again low – 16 kg while thornback ray being high – 1090 kg.

Figure 24: Porpoise with a skin lesion

Vessel 2

Set 1 (monofilament active equipped with 26 pingers Future Oceans 10 KHz) was hauled on 4 July. No bycaught cetaceans were observed.

Vessel 7

All **5 sets** have been hauled on 15/16 July. One bycaught porpoise (code 2) – lactating female, 125 cm was recorded in set 2. No bycatch was observed in the other sets including set 5 that was fitted with 25 pingers Future Oceans 10kHz. Fish catch was 100 kg of turbot and 300 kg of thornback ray.

Figure 25: Bycaught porpoise on vessel 7

Estimation of bycatch rate

In previous surveys on bycatch levels in the Black Sea different units have been used to calculate bycatch rate – individuals per 100 km of nets (Birkun Jr. et al., 2009; Mihaylov, 2010); catch per unit effort (CPUE) that is catch (individuals) divided by soaking time (hours) (Gönener and Bilgin, 2009) and individuals per kilometer of nets (Zaharieva et al., 2021) which makes comparison not possible. If we calculate bycatch rate as individuals per 100 km of nets, we get following rates:

Spring 2020

- Active nets: 7.25 porpoises and 0 bottlenose dolphins per 100 km of nets.
- Control nets: 5.43 porpoises and 1.81 bottlenose dolphins per 100 km of nets.

Summer 2020

- Active nets: 3.04 porpoises and 2.76 common dolphins per 100 km of nets.
- Control nets: 8.29 porpoises and 0 bottlenose dolphins per 100 km of nets.

Spring 2021

- Active nets: 0 porpoises and 0 dolphins per 100 km of nets
- Control nets: 29.22 porpoises and 7.3 bottlenose dolphins per 100 km of nets.

Summer 2021

- Active nets: 0 porpoises and 0 dolphins per 100 km of nets
- Control nets: 58.56 porpoises and 0 bottlenose dolphins per 100 km of nets.

If we use the alternatively used rate of individuals per kilometer, the overall mean bycatch rate during our two-year study is 0.32 which is well in line with reported bycatch rate for period 2014-2018 of 0.31 ind./km for Bulgarian Black Sea waters (Zaharieva et al., 2021). Though, if we combine data from all our bycatch study covering 2019-2021, that rate is going up to 0.48 ind./km and that is largely to higher bycatch observed in 2019 (58% of all bycatch).

During our pilot bycatch survey in 2019 we have introduced standardization of fishing effort to estimate bycatch as individuals per square kilometer of nets per day. To do that we calculated surface of nets in square kilometers and multiplied it by number of days while nets were at sea. Main reason for using square kilometers and not only kilometers is different height of used nets – from 2 to 3 m.

The formula that we use for standardized bycatch rate calculation is:

$$Bycatch\, rate = \frac{individuals}{day.km^2}$$

Given the fact that in spring 2020 catch of target species was very low that being justified by presumably short operation of nets before currents entangled these and disrupted their activity, calculated effort for that season should be taken with caution. Our estimation is that in spring 2020 only 9 (6 porpoises, 2 common and 1 bottlenose dolphin) of all 13 cetaceans found in the nets were bycaught as alive animals while the rest were dead before that. In the following season and year of the study such problem with entangled nets was not faced and consequently all cetaceans found in the nets were definitely bycaught.

Statistical analysis (nonparametric Mann-Whitney U test) has shown that there is not significant difference in bycatch rates between years 2020 and 2021 ($U=177$, $p>0.05$) and between spring and summer seasons ($U=137$, $p>0.05$). Though, ff we combine all data collected for the period 2019-2021 there is significant difference in bycatch rates between spring and summer ($U=266.5$, $p<0.05$)

Sex ratio between bycaught cetaceans was as follows:

- Bottlenose dolphin (*T. t. ponticus*) – 2 females and 1 unknown;
- Common dolphin (*D. d. ponticus*) – 1 female and 2 unknown;
- Harbour porpoise (*P. p. relicta*) – 14 males; 44 females and 14 - unknown (dropped during haul).

In spring 4 of the bycaught female porpoises were pregnant and other was lactating. In summer 14 females were lactating.

Body length of bycaught porpoises varied between 41 and 144 cm for males (mean - 115 cm) and 73 to 150 cm for females (mean - 126.88 cm); common dolphin was 159 cm and bottlenose dolphins were 180 and 211 cm.

ANALYSIS OF RESULTS FROM USE OF ACOUSTIC DETERRENT DEVICES

During the trials within the project, we used mostly PAL pingers as mitigation measure and to lesser extent Future Oceans 10 and 70kHz pingers. Comparison of obtained results, in terms of standardized bycatch rate of individuals per effort, from use of PAL pingers in 2020 and 2021 are shown in tables 4 and 5.

Table 4: Results from use of PAL pingers in 2020

active			**control**		
Vessel	**date**	**bycatch (ind./day.km^2)**	**Vessel**	**date**	**bycatch (ind./day.km^2)**
Vessel 1	10.4.2020	0	Vessel 1	10.4.2020	5,6689
Vessel 1	28.6.2020	14,88095	Vessel 1	4.7.2020	32,17503
Vessel 1	2.8.2020	3,13283	Vessel 1	23.7.2020	27,54821
Vessel 1	16.7.2020	9,92064	Vessel 1	29.7.2020	6,86813
Total		**27,93**	**Total**		**72,27**

Table 5: Results from use of PAL pingers in 2021

active			**control**		
Vessel	**date**	**bycatch (ind./day.km^2)**	**Vessel**	**date**	**bycatch (ind./day.km^2)**
Vessel 1	11.4.2021	0	Vessel 1	10.4.2021	9,448
Vessel 1	3.7.2021	0	Vessel 1	11.4.2021	26,455
			Vessel 1	13.4.2021	6,541
			Vessel 1	3.7.2021	45,7875
			Vessel 1	2.7.2021	17,806
			Vessel 1	4.7.2021	20,24619
Total		**0**	**Total**		**126,69**

Results from 2020 have shown total bycatch reduction of 61%: 27,93 in active nets versus 72,27 in control nets while those for 2021 showed 100% reduction of bycatch. Overall reduction over the two years of the study was 86%.

We have tested statistically significance of bycatch reduction by t-test, paired samples, one-sided:

Table 6: Results from t-test for significance of bycatch reduction by PAL pingers

Year	*Season*	*Vessel*	*Stand. net bycatch*	*PAL net bycatch*	**Outcome of t-test: paired samples, one-sided** Hypothesis: PAL reduces bycatch in nets set at the same time and in the same area
2020	Spring	1	5,67	0	**p = 0.003115**
2020	Summer	1	32,18	14,88	**Conclusion: Significant at 0.05 level**
2020	Summer	1	27,55	9,92	
2020	Summer	1	6,87	3,13	
2021	Spring	1	9,45	0	
2021	Spring	1	6,54		
2022	Spring	1	26,46		
2021	Summer	1	17,8	0	
2021	Summer	1	45,79		
2021	Summer	1	20,25		
		Sum	**198,56**	**27,93**	
		Bycatch reduction		**85,93%**	

Statistical test has shown that bycatch reduction value of 85.93% is significant (p = 0,003115, p < 0,05) for the entire study period of 2020 and 2021.

Trials involving Future Oceans pingers have been limited and despite results being much better compared to trials in 2019 with overall reduction of 93% in bycatch, t-test has shown it is not statistically significant (p=0,09).

Table 7: Results from t-test for significance of bycatch reduction by FO pingers in 2020-2021

Year	*Season*	*Type*	*Stand. net bycatch*	*FO net bycatch*	**Outcome of t-test: paired samples, one-sided** Hypothesis: FO reduces bycatch in nets set at the same time and in the same area
2020	Spring	10kHz	2,42	2,64	**p = 0.090746**
2020	Spring	10kHz	0,00	0,00	**Conclusion: NOT Significant at 0.05 level**
2020	Spring	70kHz	0,00	0,00	
2020	Summer	10kHz	14,88	0,00	
2021	Spring	10kHz	0,00	0,00	
2021	Summer	10kHz	20,25	0,00	
2021	Summer	10kHz	0,00	0,00	
		Sum	**37,55**	**2,64**	
		Bycatch reduction		**92,97%**	

If we combine all results from trials with Future Oceans 10 kHz model over the period 2019 to 2021, reduction of bycatch level is 24%, but t-test shows it is not statistically significant (p=0.075, p>0.05).

Table 8: Results from t-test for significance of bycatch reduction by FO 10 kHz pingers in 2019-2021

Year	***Season***	***Boat***	***Stand. net bycatch***	***FO net bycatch***	**Outcome of t-test: paired samples, one-sided** Hypothesis: FO 10 kHz reduces bycatch in nets set at the same time and in the same area
2019	Spring	2	7,12	0,00	**p = 0.075682**
	Spring	1	2,16	2,38	**Conclusion: NOT Significant at 0.05 level**
	Spring	1	0,00	2,29	
	Spring	3	0,00	8,16	
	Summer	1	35,84	55,56	
	Summer	1	103,90	86,58	
	Summer	2	16,03	0,00	
	Summer	3		16,03	
2020	Spring	1	2,42	2,64	
	Spring	2	0,00	0,00	
	Summer	1/2	14,88	0,00	
2021	Spring	2	26,46	0,00	
	Summer	2	20,25	0,00	
	Summer	7	0,00	0,00	
		Total	**229,05**	**173,64**	
	Bycatch reduction			**24,19%**	

If we combine all results from trials with Future Oceans 70 kHz model over the period 2019 to 2021, reduction of bycatch level is 40%, but t-test shows it is not statistically significant (p=0.2, p>0.05).

Table 9: Results from t-test for significance of bycatch reduction by FO 70 kHz pingers in 2019-2021

Year	***Season***	***Boat***	***Stand. net bycatch***	***FO net bycatch***	**Outcome of t-test: paired samples, one-sided** Hypothesis: FO 70 kHz reduces bycatch in nets set at the same time and in the same area
2019	Spring	1	0,00	0,00	**p = 0.203413**
	Spring	3	0,00	6,67	**Conclusion: NOT Significant at 0.05 level**
	Summer	1	78,13	74,40	
	Summer	1/5	56,69	0,00	
2020	Spring		0,00	0,00	
		Total	**134,81**	**81,07**	
	Bycatch reduction			**39,86%**	

2.2. CETACEANS' STRANDINGS

First reports of stranded cetacean during the project were received via social media on 2 April 2020 when 4 porpoises (2+2) were found by a citizen in two locations around Sozopol (Kolokita and Alepu beach) on the Southern coast. On the next two days we made monitoring of all beaches south of Burgas but no strandings were found including reported ones.

Figure 26 and 27: Stranded porpoises around Sozopol (source Facebook)

First confirmed case of bycaught stranded porpoise was on 11 April at Aheloy beach on Southern coast when a harbour porpoise with cut tail fluke was found by a volunteer. That case was followed by similar one from Varna (Northern coast) reported in social media on 27 April 2020 preceded the day before by a case on Asparuhovo beach (Varna district) with visible cuts on the side of the animal and missing muscles.

Figure 28 and 29: Stranded porpoises around Aheloy and Asparuhovo (Varna district)

On 28 April 2020, a mass stranding event was registered at Sunny beach (Nessebar municipality) when 5 porpoises were washed ashore with 2 of these with distinct bycatch signs – cut tail flukes. Since all stranded together at same site it is most likely all these were bycaught in the same string of nets.

Figures 30-34: Stranded porpoises at Sunny beach, Nessebar on 28 April 2020

On the next day – 29 April 2020, one more stranded porpoise was found at Pomorie – again with missing tail fluke indicating bycatch.

Figure 35: Stranded porpoise at Pomorie

Figure 35: Stranded porpoise at Sveti Vlas on 5 May 2020

On 30 April one more stranding was reported from Sunny beach but it was not recorded or photographed being collected by incinerator vehicle before municipal ecologists attended the site.

On 5 May 2020, another bycaught porpoise with missing tail fluke was stranded near Sveti Vlas, Nessebar.

On 9 May 2020, skeleton of Bottlenose dolphin was found on the beach near Ezerets and Shabla. It was strange that head was removed but was found nearby. All these cases coincide with the spring turbot fishing season when nets are hauled mainly just before enforcement of ban on 15 April.

Figure 37: Skeleton and skull of bottlenose dolphin at Ezerets beach, 9 May 2020

Another case of stranded porpoise that attracted media attention was on 23 May 2020 at Alepu beach, Dyuni resort. The porpoise was a pregnant female, 120 cm long and it was stranded intact. Though, on the next day holidaymaker reported that part of the porpoise was missing – head and clear cuts on its side.

Figure 38: Stranded pregnant porpoise at Alepu beach, 23 May 2020

In total 138 stranded cetaceans were recorded in 2020 – 102 porpoises; 11 bottlenose and 9 common dolphins. On 3 occasions stranded animals were identified as Delphinids while remaining 13 were unidentified.

In 2021 stranding surveys have been limited since main effort was on the bycatch monitoring. Data was collected sporadically mainly from social media and municipalities complemented with personal observations. The first stranded cetacean was registered as early as 27 February 2021 – a female bottlenose dolphin stranded on the beach of Dobrogea Camping near Shabla on the Northern coast. In total reports for 33 stranded cetaceans in 2021 were collected – 24 of these being porpoises, 6 bottlenose dolphins and 2 common dolphins and in one case species was not identified. In 6 (18%) of these cases most probable cause of death was identified to be bycatch based on missing tail or dorsal fins.

Figure 39 and 40: Stranded porpoise and bottlenose dolphin with bycatch marks – missing tail flukes

2.3. NECROPSIES, SAMPLES COLLECTION AND ANALYSIS

During the bycatch monitoring conducted within the project totally 78 cetaceans have been recorded entangled in the gillnets. 72 of these were porpoises while dolphins have been 3 of each species – bottlenose and common. 16 of these have dropped during haul of nets and were not boarded on vessels.

PILOT MICROPLASTIC STUDY

One of the main objectives of the project was to perform study on marine litter associated with cetaceans. For this purpose, a study for microplastics in the gastrointestinal tract of cetaceans was performed in collaboration with researchers from IO-BAN. Thirty stomachs have been collected from all bycaught animals: two from bottlenose dolphins and the rest from porpoises. One more stomach was collected from a porpoise that was entangled in a pound (stationary) net – Pp_Kiten_26.05.20. Two more stomachs of bycaught porpoises have been collected in 2019 and those were also handed for analysis making total to thirty-three. Some of the samples included also part of intestines. In the laboratory stomachs and intestines were weighted. The organic matter was removed with 10% potassium hydroxide /KOH/ for three weeks. The filters were examined under a stereomicroscope Olympus and each particle was categorised according to shape and colour.

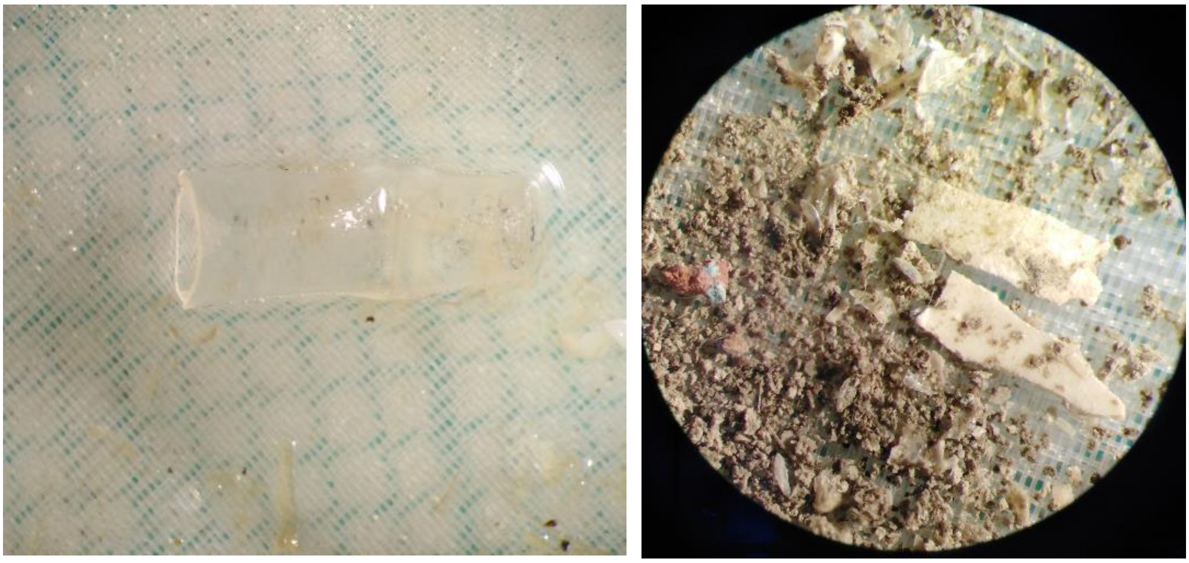

Figure 41: Examples of fragments

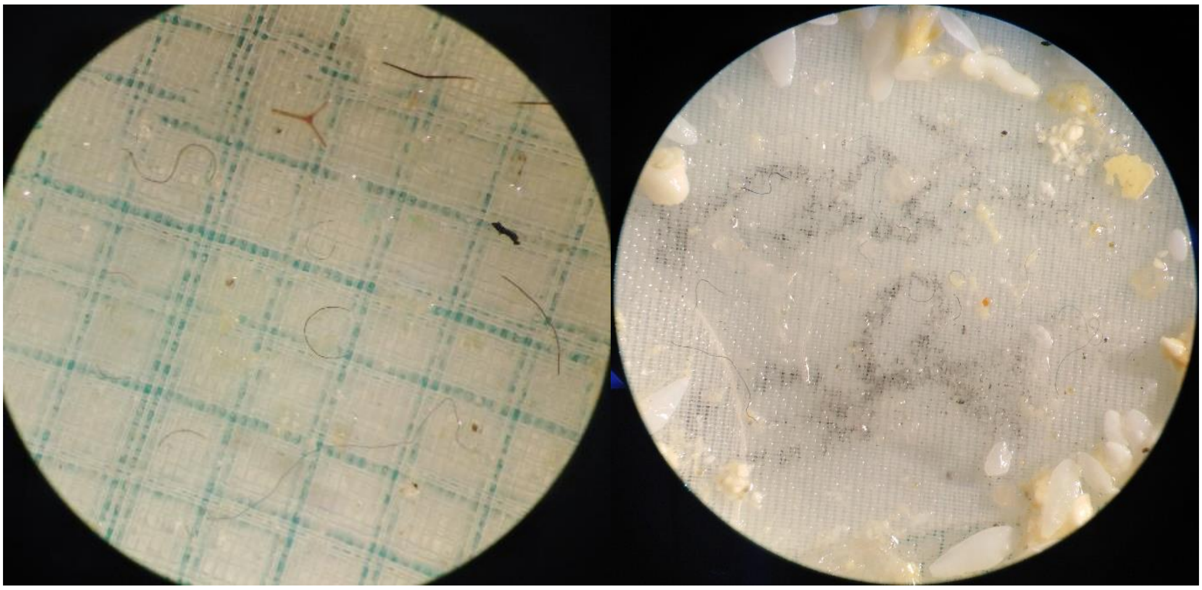

Figure 42: Examples of fibres

Results are presented in table 8.

Table 8: Results from study of microplastics in gastrointestinal tract of Black Sea cetaceans

Individual code	Species	Length	Sex	Comment	Stomach weight	Intestine weight	Body part	Type MP	Sum	MP particles per individual	Comment
Pp01_10.04.19	*Phocoena phocoena*	121	m		311,4	189,1	stomach	fibers	37	***277***	whole intestine
								fragments	10		
							intestines	fibers	204		
								fragments	26		
Pp02_10.04.19	*Phocoena phocoena*	111	m		300	173,2	stomach	fibers	76	***157***	whole intestine
								fragments	23		
							intestines	fibers	54		
								fragments	4		
Pp04_E_12.04.20	*Phocoena phocoena*	128	f		493,6		stomach	fibers	6	***27***	
								fragments	21		
Pp01_E_13.04.20	*Phocoena phocoena*	116	f	pregnant	299,5	94,3	stomach	fibers	1	***6***	partial intestine
								fragments	0		
							intestines	fibers	5		
								fragments	0		
Pp_Kiten_26.05.20	*Phocoena phocoena*	130	f		457,3		stomach	fibers	2	***6***	
								fragments	4		
Pp01_04.07.20	*Phocoena phocoena*	114	m		377,8		stomach	fibers	2	***7***	
								fragments	5		
Pp02_04.07.20	*Phocoena phocoena*	125	f		366,6		stomach	fibers	22	***22***	
								fragments	0		
Pp03_04.07.20	*Phocoena phocoena*	133	f		432,7	69,6	stomach	fibers	4	***6***	partial intestine
								fragments	0		

							intestines	fibers	2		
								fragments	0		
Pp04_04.07.20	*Phocoena phocoena*	144	m		392,7		stomach	fibers	0	***2***	
								fragments	2		
Pp06_04.07.20	*Phocoena phocoena*	136	f		439,2		stomach	fibers	2	***3***	
								fragments	1		
Pp08_04.07.20	*Phocoena phocoena*	126	f		639,3		stomach	fibers	2	***2***	
								fragments	0		
Pp10_04.07.20	*Phocoena phocoena*	124	f		377,4		stomach	fibers	3	***3***	
								fragments	0		
Pp12_04.07.20	*Phocoena phocoena*	117	m		355,6	72,1	stomach	fibers	1	***12***	partial intestine
								fragments	0		
							intestines	fibers	11		
								fragments	0		
Pp01_16.07.20	*Phocoena phocoena*	135	f		456,4		stomach	fibers	10	***10***	
								fragments	3		
Pp02_16.07.20	*Phocoena phocoena*	130	f		474,1		stomach	fibers	26	***26***	
								fragments	0		
Pp01_23.07.20	*Phocoena phocoena*	133	f		484,8		stomach	fibers	1	***4***	
								fragments	3		
Pp02_23.07.20	*Phocoena phocoena*	127	m		337,6		stomach	fibers	1	***1***	
								fragments	0		
Pp03_23.07.20	*Phocoena phocoena*	116	f		357,3		stomach	fibers	23	***24***	
								fragments	1		
Pp04_23.07.20	*Phocoena phocoena*	128	f		474,3		stomach	fibers	0	***1***	
								fragments	1		
Pp05_23.07.20	*Phocoena phocoena*	101	f		293,1		stomach	fibers	3	***3***	
								fragments	19		
Tt 01_10.04.21	*Tursiops truncatus*	202	f		1140,5		stomach	fibers	13	***24***	
								fragments	11		
Pp02_10.04.21	*Phocoena phocoena*	132	f	pregnant	349,1	69,6	stomach	fibers	8	***20***	partial intestine
								fragments	3		
							intestines	fibers	7		
								fragments	2		
Pp03_10.04.21	*Phocoena phocoena*	130	f	pregnant	257,8		stomach	fibers	8	***13***	
								fragments	5		
Pp01_11.04.21	*Phocoena phocoena*	129	f	pregnant	358,2	68,7	stomach	fibers	3	***11***	partial intestine
								fragments	4		
							intestines	fibers	4		
								fragments	0		
Pp02_13.04.21	*Phocoena phocoena*	121	f		366,3		stomach	fibers	8	***9***	
								fragments	1		
Tt01_13.04.21	*Tursiops truncatus*	211	f		522,5		stomach	fibers	6	***9***	
								fragments	3		
Pp04_2.07.21	*Phocoena phocoena*	126	f	lactating	419,8	96,2	stomach	fibers	10	***20***	partial intestine
								fragments	1		

							intestines	fibers	9		
								fragments	0		
Pp05_2.07.21	*Phocoena phocoena*	129	f		394,7		stomach	fibers	0	***2***	
								fragments	2		
Pp07_2.07.21	*Phocoena phocoena*	112	m		259,5		stomach	fibers	2	***4***	
								fragments	2		
Pp01_3.07.21	*Phocoena phocoena*	115	f	lactating	265,5	143,2	stomach	fibers	2	***14***	whole intestine
								fragments	2		
							intestines	fibers	7		
								fragments	3		
Pp01_4.07.21	*Phocoena phocoena*	123	f	lactating	371,4	64	stomach	fibers	4	***11***	partial intestine
								fragments	0		
							intestines	fibers	4		
								fragments	3		
Pp06_4.07.21	*Phocoena phocoena*	133	f	lactating	388,5	82,7	stomach	fibers	10	***16***	partial intestine
								fragments	4		
							intestines	fibers	2		
								fragments	0		
Pp10_4.07.21	*Phocoena phocoena*	117	m	lactating	274,7	38,6	stomach	fibers	1	***6***	partial intestine
								fragments	0		
							intestines	fibers	5		
								fragments	0		

In twelve (36%) of the collected samples not only stomach but also part of whole intestine was collected. Results of these samples have shown that in half of the cases more microplastics were found in the intestine. Two samples from spring 2019 were with considerably higher number of microplastics – 157 and 277 while for the samples from 2020-21 the peak was 27. Even without the extreme value from spring 2019 average, values for spring samples were higher than those for summer ($p<0,05$). Fibres were dominant type of microplastics found: 611 (78%) while fragments were 169 (22%). No macro plastics have been found in any of the samples and sizes were in the range: 5 - 25 mm. Following colours of microplastics were observed in the samples: brown, blue, black, pink, grey, green, orange, red, white, lilac and transparent. The most frequent were blue, grey and transparent.

Analysis of stomach contents was complemented with study of prey items by Dr. Marina Panayotova of IO-BAS, Varna. Three of the stomachs were completely empty. Based on otoliths following fish species have been identified in the remaining 30 samples: Whiting (*Merlangius merlangus*), Sprat (*Sprattus sprattus*), Horse mackerel (*Trachurus mediterraneus*), Pontic shad (*Alosa pontica*), Anchovy (*Engraulis encrasicolus*), Gobies (Gobidae). The most abundant and dominant prey item was

Whiting (missing in only eight samples – 27%). Both bottlenose dolphins prey contents were whiting and gobies. Pontic shad, anchovy and horse mackerel were represented by single specimens only. Maximum prey number was more than 61, found in a female harbour porpoise. All identified prey items are presented in table 9.

Table 9: Prey contents in stomachs of bycaught cetaceans

№	Code	Prey, age	Couple otoliths	Sum prey
1	Pp01_10.04.19	Sprattus sprattus 0+	2	>35
		Merlangius merlangus 0+	>32	
		Merlangius merlangus 2	1	
2	Pp02_10.04.19	Sprattus sprattus 0+	4	4
3	Pp01_E_13.04.20	Gobidae	1	13
		Sprattus sprattus 1	2	
		Merlangius merlangus 0+	2	
		Merlangius merlangus 2-3	8	
4	PpKiten_26.05.20	Sprattus sprattus 2	1	14
		Merlangius merlangus 0+	11	
		Merlangius merlangus 2	2	
5	Pp01_04.07.20	Merlangius merlangus 0+	2	8
		Merlangius merlangus 1+	5	
		Merlangius merlangus 2	1	
6	Pp02_04.07.20	Merlangius merlangus 0+	2	3
		Merlangius merlangus 1+	1	
7	Pp03_04.07.20	Sprattus sprattus 0-1	8	8
8	Pp04_04.07.20	Sprattus sprattus 1	6	16
		Merlangius merlangus 0+	9	
		Merlangius merlangus 3-5	1	
9	Pp06_04.07.20	Trachurus mediterraneus	1	12
		Merlangius merlangus 0+	9	
		Merlangius merlangus 2	2	
10	Pp08_04.07.20	Gobidae 0+	1	2
		Sprattus sprattus 0+	1	
11	Pp10_04.07.20	Sprattus sprattus 0+	4	4
12	Pp12_04.07.20	Alosa pontica 1	1	7
		Sprattus sprattus 0+	2	
		Merlangius merlangus 0+	4	
13	Pp01_16.07.20	Sprattus sprattus 0+	1	3
		Merlangius merlangus 0+	1	
		Merlangius merlangus 1	1	
14	Pp02_16.07.20	Sprattus sprattus 0+	4	8
		Merlangius merlangus 0+	4	
15	Pp01_23.07.20	Merlangius merlangus 0+	~21	~21
16	Pp02_23.07.20	Sprattus sprattus 0+	4	4
17	Pp03_23.07.20	Merlangius merlangus 0+	>60	>61
		Merlangius merlangus 2-3	1	

18	Pp04_23.07.20	Sprattus sprattus 0+	2	2
19	Pp05_23.07.20	Merlangius merlangus 0+	~25	~25
20	Tt 01_10.04.21	Gobidae	2	16
		Merlangius merlangus 0+	10	
		Merlangius merlangus 1	4	
21	Pp02_10.04.21	Merlangius merlangus 0+	1	4
		Merlangius merlangus 2	2	
		Merlangius merlangus 2-3	1	
22	Pp03_10.04.21	Merlangius merlangus 1	1	1
23	Pp01_11.04.21	Sprattus sprattus 0+	3	10
		Merlangius merlangus 0+	6	
		Merlangius merlangus 2	1	
24	Pp02_13.04.21	Merlangius merlangus 0+	1	1
25	Tt01_13.04.21	Gobidae	3	9
		Merlangius merlangus	6	
26	Pp04_2.07.21	Sprattus sprattus 0+	3	3
27	Pp05_2.07.21	Engraulis encrasicolus 2	1	1
28	Pp07_2.07.21	Merlangius merlangus 0+	1	1
29	Pp01_4.07.21	Sprattus sprattus 0+	4	5
		Merlangius merlangus 0+	1	
30	Pp10_4.07.21	Sprattus sprattus 2-3	1	1

In addition, tissue samples have been collected from some animals for performing histopathological study (annex 1). Collected samples are presented in table 10.

Table 10: Collected tissue samples from bycaught cetaceans

Date	Species	Length	Sex	Tissue samples
6.7.2019	*Phocoena phocoena*	114	f	lung, liver, kidney, heart, muscle
6.7.2019	*Phocoena phocoena*	113	m	lung, liver, kidney, heart, muscle, testicle
6.7.2019	*Phocoena phocoena*	117	m	skin
12.4.2020	*Phocoena phocoena*	128	f	liver, kidney, heart, uterus, lung
13.4.2020	*Phocoena phocoena*	116	f	uterus, liver, kidney
4.7.2020	*Phocoena phocoena*	126	f	ovary, skin
4.7.2020	*Phocoena phocoena*	114	m	liver
4.7.2020	*Phocoena phocoena*	144	m	kidney
23.7.2020	*Phocoena phocoena*	133	f	skin
23.7.2020	*Phocoena phocoena*	122	f	skin
10.4.2021	*Tursiops truncatus*	202	f	heart, kidney, liver, lung
10.4.2021	*Phocoena phocoena*	132	f	spleen
11.4.2021	*Phocoena phocoena*	129	f	liver, lung, kidney
13.4.2021	*Phocoena phocoena*	121	f	heart, muscle, lung
13.4.2021	*Tursiops truncatus*	211	f	skin
2.7.2021	*Phocoena phocoena*	129	f	kidney, liver, lung
3.7.2021	*Phocoena phocoena*	115	f	liver, lung
4.7.2021	*Phocoena phocoena*	123	f	skin

Figure 43: Necropsy of porpoise in spring 2020

Figure 44: Necropsy of Black Sea bottlenose dolphin in spring 2021

Tissue samples from organs were handed to Dr. Kiril Dimitrov of Trakia University's Vet Faculty in Stara Zagora for histopathology studies. Results of that study are presented as Annex 1 to the report.

Figure 45: Pregnant porpoise with fetus in spring 2021

2.4. PRESENTING PROJECT RESULTS

Project results and especially bycatch rates and effect of used acoustic deterrent devices have been presented at different events. These are listed below:

- First Meeting of the ACCOBAMS/ASCOBANS Joint Bycatch Working Group (online) - 10-12 February 2021;

Figure 46: Presenting the project results during First meeting of ACCOBAMS/ASCOBANS Joint Bycatch Working Group, 11 February 2021

- Meeting (online) of GFCM Working Group on Fishing Technology (WGFIT): 8-9 April 2021;

During these meetings results from trials held within the project only in 2020 were presented and complemented with data from 2019 pilot when only Future Oceans pingers were tested. One of the results from presenting project results at GFCM WGFIT was drafting a concept note for pilot project on bycatch mitigation in the Black Sea by GFCM with potential replication of PAL trials at other sites/countries in the Black Sea covering Ukraine, Romania, Bulgaria and Turkey. Realization of such project is important for obtaining stronger results in terms of statistics. On the other hand specifically for Bulgaria it is essential to not lose gained momentum and build on established cooperation with fishermen.

Full results of trials conducted in the scope of the current project were further presented at the following fora:

- Ninth meeting (online) of the GFCM Working Group on the Black Sea (WGBS): 28-30 July 2021;
- Workshop on bycatch mitigation in the Black Sea that was organized within CONCETA project in Constanta: 25-30 October 2021;
- 14th meetings of ACCOBAMS Scientific Committee held in Monaco: 22-26 November 2021.

Figure 47: Presenting project results to CONCETA workshop

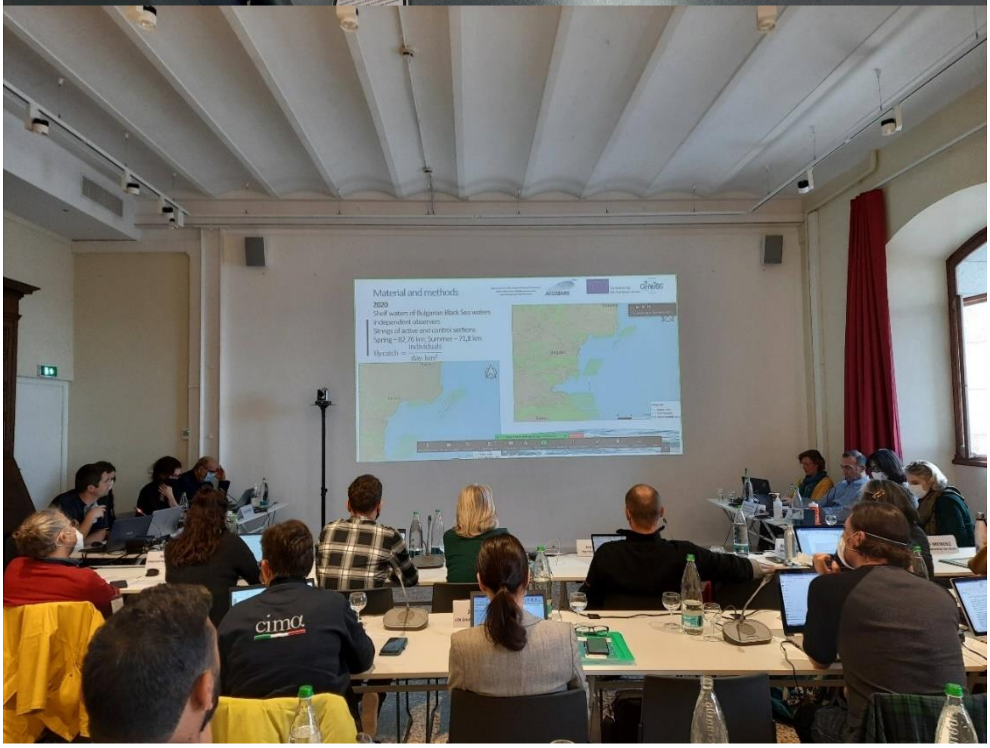

Figure 48: Presenting project results at ACCOBAMS Scientific Committee meeting

3. DIFFICULTIES ENCOUNTERED AND MEASURES TAKEN TO OVERCOME PROBLEMS

During the project implementation following difficulties have been encountered:

- COVID-19 pandemic and introduced emergency situation in Bulgaria enforced two of the fishermen operating smaller vessels from Primorsko in Southern sector to cancel fishing efforts in 2020;
- Bad weather conditions in spring 2020 caused improper operation of nets but extension of project helped to implement more trials.
- In summer 2020 two of the strings have been broken by trawler/s causing loss of 3 PALs and certain number of nets for the fisherman. That was a major problem concerning safety of nets and attached pingers. Complaint was submitted to Fisheries and Aquaculture Executive Agency but that has not helped to get back the missing PAL devices.
- Smaller vessels cannot take observers onboard thus limiting independency of observations and relying on self-reporting.
- One of the fishermen ceased operation and has sold his vessel but thanks to agreement with other fisherman he still continued using his nets and attached FO pingers to these in 2021. As nets were very worn it was their last use.

4. CHANGES INTRODUCED IN THE IMPLEMENTATION

Two important changes have been made – extension of project duration from 1 to 2 years and change of used acoustic deterrent devices model. Major change was substituting purchase of 100 Future Oceans pingers with free trial use of 40 PALs by F^3 Maritime Technology Germany on the condition to pay for transport costs and maintenance of these devices. Due to larger declared interest and involvement of more fishermen in the trial, additional 31 Future Oceans 70 KHz pingers were purchased in spring 2020. In the end of March 2020, 40 more PALs were allowed by F^3 Maritime Technology thus altogether 80 PALs were available for the trial in summer 2020. Due to damages and losses during the trial in 2020 number of available PALs in 2021 was lower – 66 but that was still sufficient for the trial and equipping one of used long strings by vessel 1.

5. ACHIEVEMENTS/RESULTS

During the project implementation following results have been achieved:

- Completed **bycatch monitoring of 243 880 m bottom set gillnets** during two years (2020 and 2021) and three seasons (spring, summer and autumn) of turbot fishing;
- Results from tested acoustic deterrent devices (**PAL pingers**) have shown **decrease of bycatch of 86%** to be statistically significant;
- Collected **thirty-three stomachs** of bycaught cetaceans (31 of porpoises and 2 of bottlenose dolphin) used for **pilot study of microplastics in gastrointestinal tract of Black Sea cetaceans**;
- **Tissue samples** for histological studies have been collected from eighteen bycaught cetaceans – two bottlenose dolphin and sixteen porpoises;
- Performed stranding surveys for collection of additional data on bycatch and identification of illegal, unreported and unregulated (IUU) fishing.

6. CONCLUSIONS AND RECOMMENDATIONS

Bycatch is the most significant source of human-induced mortality for the Black Sea harbour porpoise (Birkun 2002). In recent years several efforts to measure bycatch level of cetaceans in the Black Sea were made using several approaches: scientific literature review, questionnaire surveys, on-board monitoring (Tonay 2016; Birkun et al., 2014; CeNoBS, 2021). All of these studies had similar conclusion – bycatch rate of cetaceans and especially Black Sea harbour porpoise is exceeding sustainable levels of 1-1.7% of population abundance, adopted by international organisations. Finding reliable solution for mitigation of bycatch is of utmost importance to ensure good conservation status of Black Sea cetaceans. On the basis of conducted trials and obtained results we can make following conclusions and recommendations:

- **Bycatch** of **harbour porpoise** in **Bulgarian waters** is **exceeding sustainable level of 1.7% of abundance estimation**. Line-transect distance sampling vessel surveys conducted in Bulgarian territorial waters in 2020 and 2021 have shown following numbers: spring 2020 – 4889 (CV=21.56%); summer 2020 – 991 (CV=42.62%); spring 2021 – 3023 (CV=28.52%) and summer 2021 – 3559 (CV=45.59%). Conducted bycatch monitoring in 2020 and 2021 covered respectively 3.2% and 2.4% of licensed vessels for turbot fishing. Recorded bycatch from these vessels was 43 and 29 porpoises in 2020 and 2021 respectively. Taking into account season shows we recorded as bycatch following share of estimated abundance: spring 2020 - 0.1%; summer 2020 – 3.7%; spring 2021 – 0.3% and summer 2021 – 0.6%. Even applying the most conservative extrapolation of that rate to entire national turbot fishing fleet would produce strikingly high overall bycatch rate.

- Results from trials including PAL pingers as mitigation measure to minimize bycatch of Black Sea harbour porpoise have shown that observed **decrease of bycatch rate of 86% is statistically significant (p<0.05). That result is similar to achieved decrease with PAL devices in the Baltic Sea.** Better result for use of pingers as mitigation measure in the Black Sea was previously reported only for Dukane NetMark (Gonener and Bilgin, 2009) but that model is no longer produced.
- Positive results obtained by use of PAL pingers have been the main reason for selecting that model for trials in Sinop, Turkey within CONCETA project that is on-going. Results obtained from trials conducted in that area are essential to confirm effectiveness of these devices.
- **Number of bycaught individuals** was found to be **correlated** significantly (r=0.606, p<0.05) only with **length of net strings** and not with soaking time and depth. Standardized bycatch (ind./effort) has not shown positive correlation with any of these factors.
- Continued trials for deployment of Future Oceans 10kHz and 70kHz pingers have shown much better results in 2020 and 2021 – **93% decrease but that was not statistically significant (p>0.05)**. One probable reason for better results in the current study period compared to 2019 could be associated with use of these models in shorter strings in the current trials.
- On the basis of the obtained results, **we can recommend use of PAL pingers as an effective mitigation measure** for lowering bycatch of Black Sea harbour porpoise – an Endangered species listed in IUCN Red List.
- **Higher bycatch rates in early summer** coincide with **the highest density** of the **Black Sea harbour porpoise in Bulgarian waters**. Extension of

turbot fishing ban until 1st or 10th July should be considered as additional mitigation measure for **lowering bycatch level**.

7. SUMMARY

This project's main goal was to continue tests of pingers as mitigation measures for lowering levels of cetacean bycatch in turbot fishery that is considered most important threat to Black Sea Harbour Porpoise (Endangered subspecies in IUCN Red List). 7 fishing vessels agreed to participate in trials of pingers in bottom set gillnets used for turbot fishing. Onboard monitoring by independent observers was organized for 4 of the fishing vessels while other two provided data on bycatch.
In total 243 880 m of bottom set gillnets have been monitored. 78 cetaceans have been recorded as bycatch in the gillnets – 72 Black Sea harbour porpoises, 3 Black Sea bottlenose dolphins and 3 Black Sea common dolphins. 2 water birds were also found in the nets – 1 Coot and 1 Moorhen. Pregnant porpoises were found in spring and lactating in summer. Overall mean bycatch rate was 0.32 ind./km net while standardized bycatch rate of individuals per effort (square kilometers*days) varied between 0 and 45.79 (mean – 7.01). Trials of PAL pingers over two years have shown reduction of bycatch with 86% that was statistically significant ($p<0.05$). Pilot study of microplastics in gastrointestinal tract of Black Sea cetaceans (harbour porpoise and bottlenose dolphin) was made revealing existence of fibres and fragments (size = 5 - 25 mm) in all samples (1-277 items). Complementary study of prey items has revealed large dominance of whiting (*Merlangius merlangus*) found in 73% of stomachs. Histological study revealed that almost half of the bycaught cetaceans were suffering of pneumonia.

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ANNEX 1: HISTOPATHOLOGICAL STUDY

REPORT ON POSTMORTEM EXAMINATIONS FOR MONITORING HEALTH STATUS OF BYCAUGHT BLACK SEA CETACEANS IN BULGARIAN WATERS

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Introduction

Black Sea is an isolated enclosed water basin located between Asia and Europe and enclosed between Bulgaria, Georgia, Romania, Russia, Turkey, and Ukraine. It is characterized by lower salinity (14-16‰) and huge anoxic underlayer below depth of 100-150 m. Despite that Black Sea supports an active, dynamic and yet fragile marine ecosystem. At the top of the food chain is the marine megafauna represented by two species of dolphin (common – *Delphinus delphis ponticus* and bottlenose – *Tursiops truncatus ponticus*) and the harbour porpoise (*Phocoena phocoena relicta*). Cetaceans are both considered as vulnerable and biomarker species reflecting the condition of the ecosystem.

Environmental issues and the protection and rehabilitation of the Black Sea are attracting greater interest leading to signing of Black Sea Bucharest Convention. In the 20th century, Black Sea suffered major and very evident decline caused by the human activities. The sea has been overexploited by fishing, tourism, mineral extraction, marine transport and military activities.

One of the main environmental challenges for the Black Sea is the conservation of Black Sea biodiversity and habitats many of which are protected by national and international legislation (ex. EU Bird Directive; EU Habitats Directive; Convention on Migrating Species; ACCOBAMS, etc.). Study of health status of cetaceans is complicated and strandings provide opportunity for that but usually carcasses are not fresh. Bycatch monitoring aboard fishing vessels provide good opportunity for post mortem investigations as major tool for diagnosis and health monitoring of cetaceans.

Aims

The main objective of this study is to perform postmortem investigation of bycaught cetaceans, to collect tissue samples for histopathology in order to monitor health status and to describe the common changes associated with that cause of death.

Material and methods

The recent study was conducted during the period from April 2019 until July 2021 as part of project “Monitoring and mitigation of cetaceans’ bycatch in Bulgarian waters” MoU14/2019 Samples were collected following appropriate licenses and regulations from the relevant authorities (License No 772/13.02.2019 by Ministry of Environment and Water). A total of (n=18) individuals were necropsied and samples were collected from these. 16 of these were Black Sea harbour porpoises and 2 were Black Sea bottlenose dolphins. Both bottlenose dolphins were females while porpoises were respectively 12 females and 4 males. Postmortem examination and collection of tissue samples were conducted following the currently established best practices on cetacean post mortem investigation and tissue sampling. After extraction a total of n=92 tissue samples were fixed in 10% phosphate-buffered formalin solution, dissected and embedded in paraffin wax using standard techniques processed for histopathology at the Pathologic Anatomy Unit, Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora. Then sections of 5 μm thickness were prepared, stained with hematoxylin and eosin (H&E) and assessed under a light microscope. Representative microphotographs were obtained using a digital microscope camera. In addition, teeth samples were collected for further age determination and stomach content samples for diet analysis, marine litter and micro-plastics.

Results and Discussion

Gross findings

All carcasses were retrieved from bottom-set gillnets for turbot (stretched mesh size = 400 mm) as bycatch. The main external features are described in Table 1.

Table. 1. Summary of identification, basic body measurements and external gross pathology finding of bycaught cetaceans from the Bulgarian Black Sea waters, 2019 -2021

Id #	Species	Date	Sex	Body length	Reproductive state	External examination
01	P.p.	06.07.19	F	114	-	-
11	P.p.	06.07.19	M	117		Skin lesions
38	P.p.	06.07.19	M	113	-	-
04	P.p.	12.04.20	F	128	-	-
01	P.p.	13.04.20	F	114	pregnant	-
01	P.p.	04.07.20	M	114	-	Lesion on dorsal fin
04	P.p.	04.07.20	M	144	-	-
08	P.p.	04.07.20	F	122	-	Skin lesions
01	P.p.	23.07.20	F	133	-	Skin lesions
08	P.p.	23.07.21	F	122	lactating	Skin lesions
01	T.t.	10.04.21	F	202	-	-
01	T.t.	13.04.21	F	211	-	Skin lesions
01	P.p.	03.07.21	F	115		
01	P.p.	04.07.21	F	123	lactating	Skin lesions
05	P.p.	02.07.21	F	129	-	-
02	P.p.	10.04.21	F	132	pregnant	-
01	P.p.	11.04.21	F	129	-	-

02	P.p.	13.04.21	F	121	-	-

Grossly the observed skin lesions in both species mainly appeared as focal to multifocal and coalescing patchy stippled skin discolorations with gray or black colour – hyperpigmentation often described as "tattoo skin lesions". Single individuals from both species demonstrated and other type of lesions whose shape was as focal variable circular or targetoid, often raised, and centrally ulcerations defined as ulcerative dermatitis, which could be referred either as "morbilli-like lesions", "fresh water skin disease lesions" or "dermatitis by other cause". Only one purpoise was retrieved with net marks with grooves and bruises due to entanglement in the gill net.

After stripping aimals' skin and blubber there were no visible lesions, discolorations or signs of parasite infestation.

Nutritional condition state for all of the animals for both species was estimated as very good according to their blubber thickness, lipid composition and back muscle mass. The outlining features of the carcasses appeared convex on cranial and round on caudal perspective, respectively visible to the skull and lateral to the dorsal fin. There subcutaneous, pleural and other visceral fat were present and blubber layers were thick.

Examining the internal organs also has not revealed any specific changes, in general they appeared mildly congested and fresh despite the initial postmortem changes. The most common gastric findings were empty alimentary tract, otoliths, fish vertebrae, partially digested or undigested fresh prey. No foreign bodies, macro plastics, parasites or parasitic lesions were seen during exploration of the gastrointestinal tract. In just a few cases regurgitated fish was observed in the mouth. Thoracic cavity and cranium were intact no bone fractures or significant traumatic lesions were detected during investigation of the musculoskeletal system.

The development of all fetuses found in the uterus of pregnant porpoises corresponded to advanced gestation period.

Cranium, brain, mid ear had not been thoroughly examined due to lack of technical time while performing post mortem examination on board site of the fishing vessel.

All carcass remains were disposed by throwing them overboard. Unfortunately, no actions to assure carcass sinking were able to take place.

Histopathology

Microscopic observation of skin samples from both cetacean species revealed a multifocal, mild to moderate hydropic degeneration of stratum spinosum keratinocytes, with simultaneous occurrence of round, eosinophilic, glassy structures (intracytoplasmic eosinophilic inclusion bodies) compatible with B-type poxviral inclusions (Guarnieri bodies). Such lesions are often described as tattoo-like skin lesions (resp. tattoo-like skin disease). The overlying stratum corneum was usually eroded with areas with mild orthokeratotic hyperkeratosis and melanosis, occasionally containing Guarnieri bodies (Fig. 1 and Fig. 2). Pox virus infections generally do not affect the overall health of cetaceans, however regarding to some studies prevalence of tattoo-like skin lesions could be related to compromised environmental conditions and consequently general health of the affected individuals.

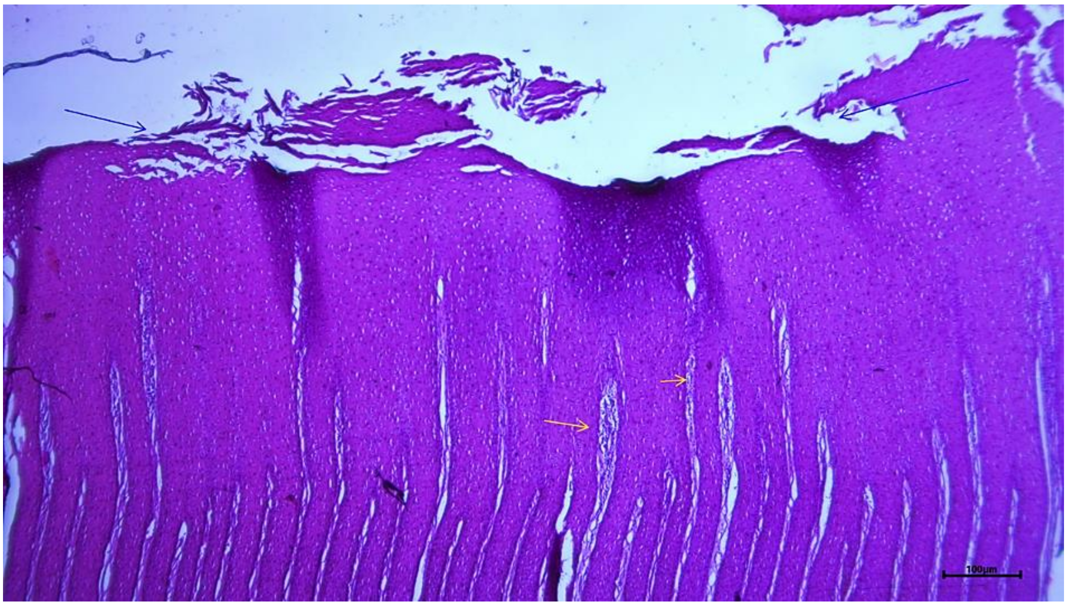

Fig. 1: Erosion, hyperkeratosis and interfacial dermatitis, tattoo-like skin lesion, skin, P.p., H&E, bar = 100µm

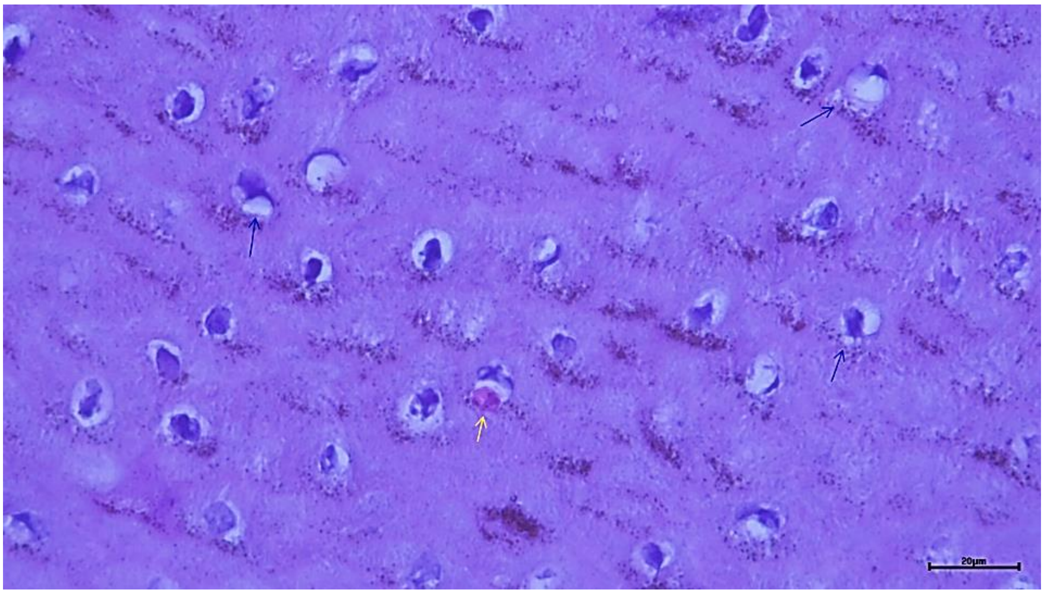

Fig. 2. Poxviral eosinophilic intracytoplasmic inclusions - Guarnieri bodies and hypermelanosis, tattoo-like skin lesions, skin, T.t., H&E, bar = 20 µm

The other most distinctive skin lesions resembled the features of either dark focal skin disease (dFSD, tattoo-like skin) or ulcerative focal skin disease (uFSD). Histologically uFSD lesions had abrupt transition from the relatively normal epidermis to area with marked acantosis at the periphery of the ulcer. In the central area of the lesion there were mild hydropic changes, karyopyknosis, eosinophilia in the deeper epidermal layers and closer to the epidermal-dermal junction together with marked hyperemia in the subcutis (Fig. 3.).

The dFSD lesions had the appearance of patchy areas with mild orthokeratotic hyperkeratosis and marked fibrosis and hyperpigmentation of the deeper epidermal layers (Fig. 4).

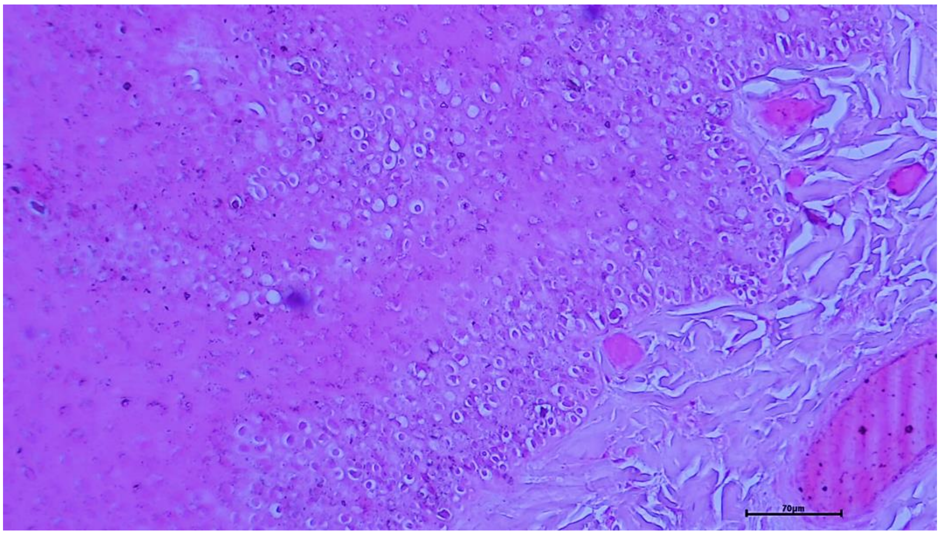

Fig. 3. Ballooning degeneration and karyopyknosis, skin, P. p., H&E, bar = 70 µm

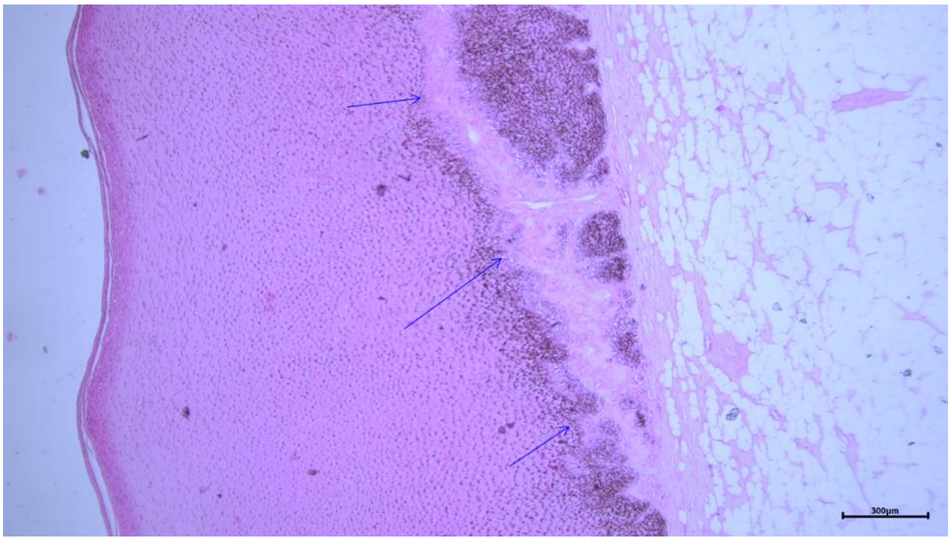

Fig. 4. DFSD lesion with distinct scarring and hypermelanosis, skin, T.t, H&E, bar = 300 µm

Tattoo-like skin lesions are considered a common finding in adolescents and young individuals while dFSD and uFSD are more common in mature individuals. The presence of tattoo-like skin lesions indicates a healthy population capable of overcoming this type of poxvirus infection. While for the other type of lesions the causes can differ and be associated with a number of pathogenic bacteria, fungi and viruses and usually their exact etiology cannot be determined. Their higher frequency is usually due to anthropogenic pollution and other factors such as the lower salinity of the Black Sea.

In histopathology of lungs all of the samples showed marked congestion, slight oedema, emphysema with foci of alveolar wall rupture (Fig. 5.) and microhemorrhages. Concurrently with these findings in almost half of the cases (44%) lungs had pneumonic lesions either as scant focal to coalescing broncho-interstitial foci (Fig. 6.), interstitial pneumonia, scarring or chronic granulomatous lesions characteristic for parasitic pneumonia (Fig. 7.)

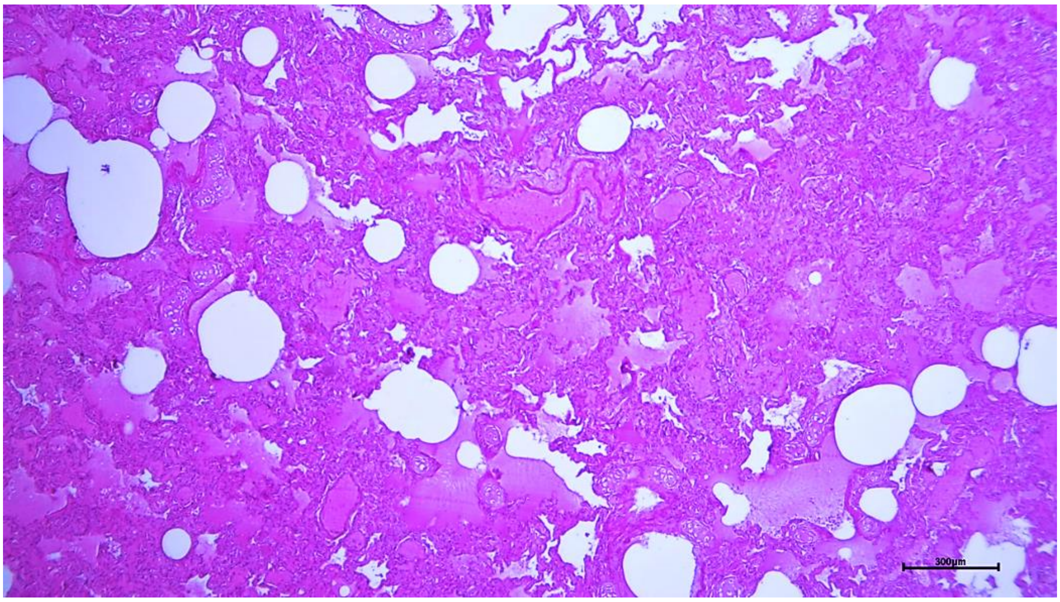

Fig. 5. Congestion, oedema, emphysema with ruptured alveoli, P.p., lung, H&E, bar = 300 µm

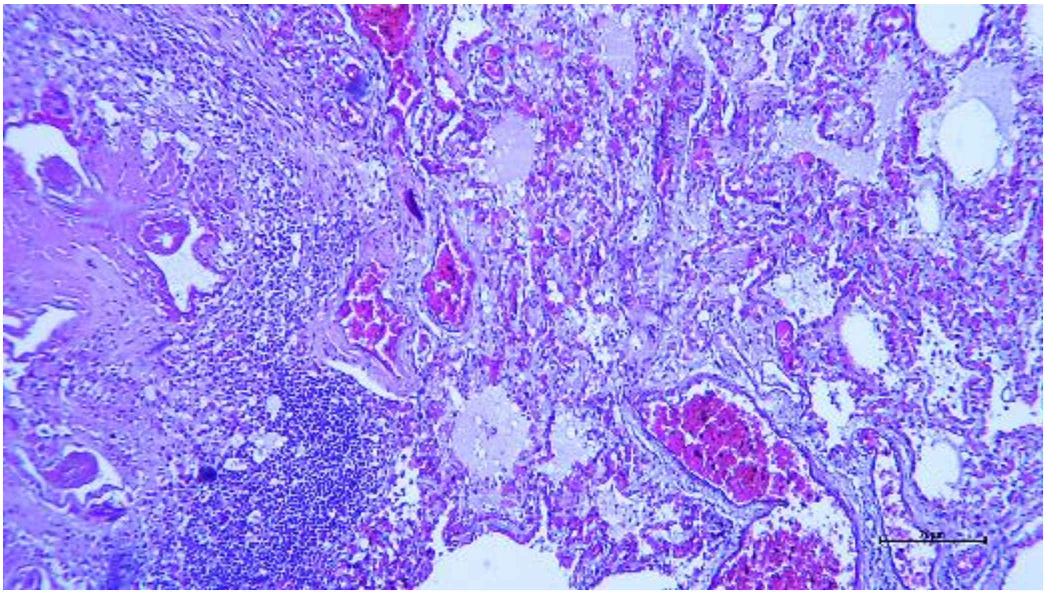

Fig. 6. Broncho-interstitial pneumonia, scarring, congestion and slight oedema, lung, T.t., H&E, bar = 200 µm

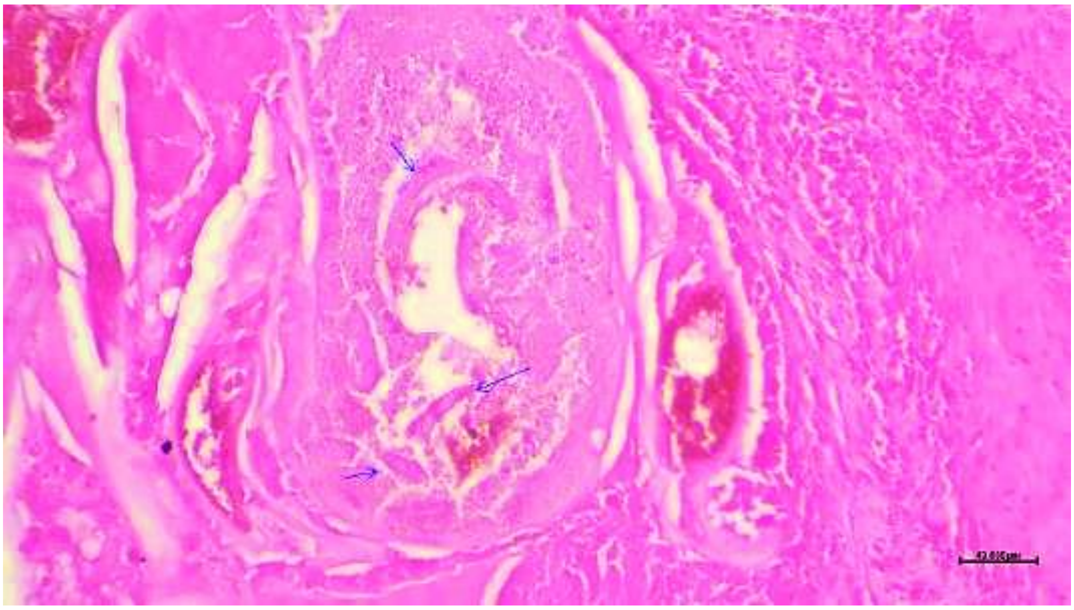

Fig. 7. Nodular parasitic granulomatous lesion containing nematodes, lung, P.p., H&E, bar = 50 µm

The pneumonia in small cetaceans is considered as a common disease. It is often the result of bacterial, viral infections and parasitic infestations. Despite the high incidence of the disease, most individuals get sick without much damage to their general health. On the other hand, the increase in the incidence of pneumonia and the predominance of a causative agent or factor for its development can be used as a clear marker for the health status of the population.

Samples from kidneys for both cetacean species revealed common findings of severe congestion, microhemorrhages, degeneration of proximal tubule, cortical emphysema and autolysis (Fig. 8)

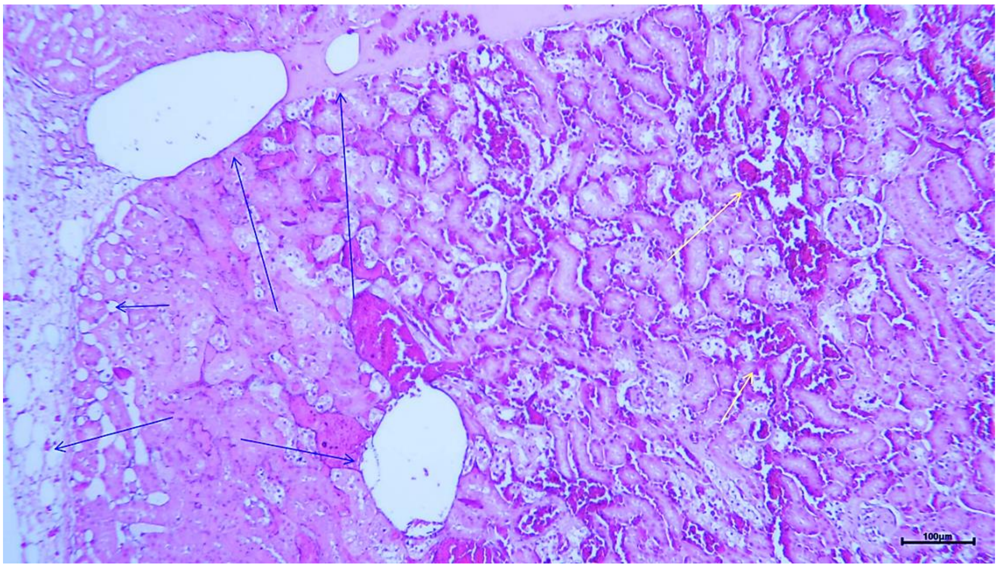

Fig. 8. Kidney with tissue emphysema, congestion and microhemorrhages, kidney, T.t., H&E, bar = 100 µm

The findings in liver samples also had similar pattern in all of the tested animals. Despite of the clearly advanced autolysis, liver were found either congested and occasionally with large multifocal to coalescing clear spaces due to the accumulation of gasses (Fig. 9.) No evidence for dissemination and association of putrid microorganisms with the gas cavities was found.

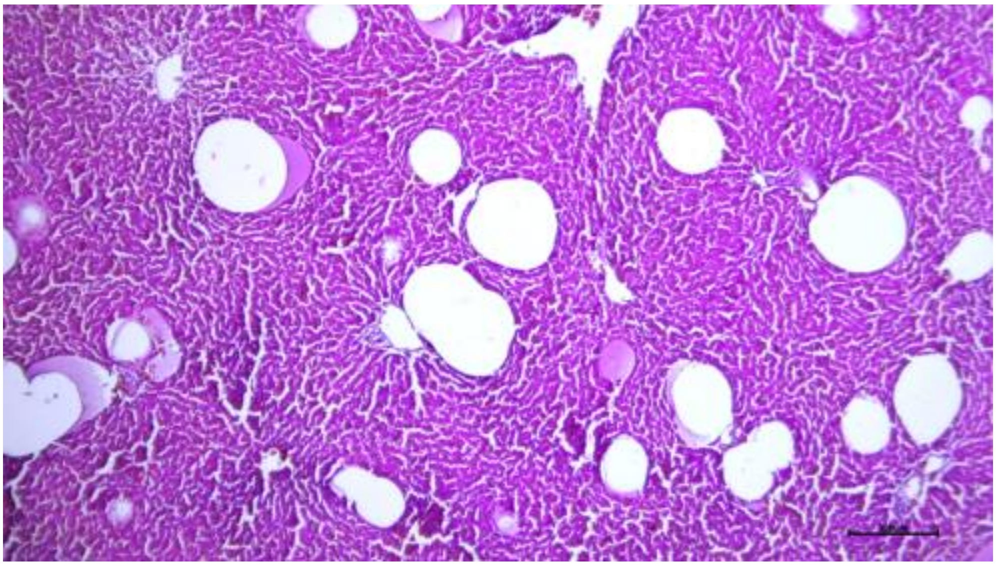

Fig. 9. Congested and emphysematous liver, P.p., H&E, bar = 300 µm

Tissue samples from heart had no significant microscopic lesion despite the overall congestion and the usual post mortem autolysis. However, the samples from skeletal musculature of bycaught P.p. showed evidence of congestion, degeneration, intermuscular oedema and emphysema (Fig. 10).

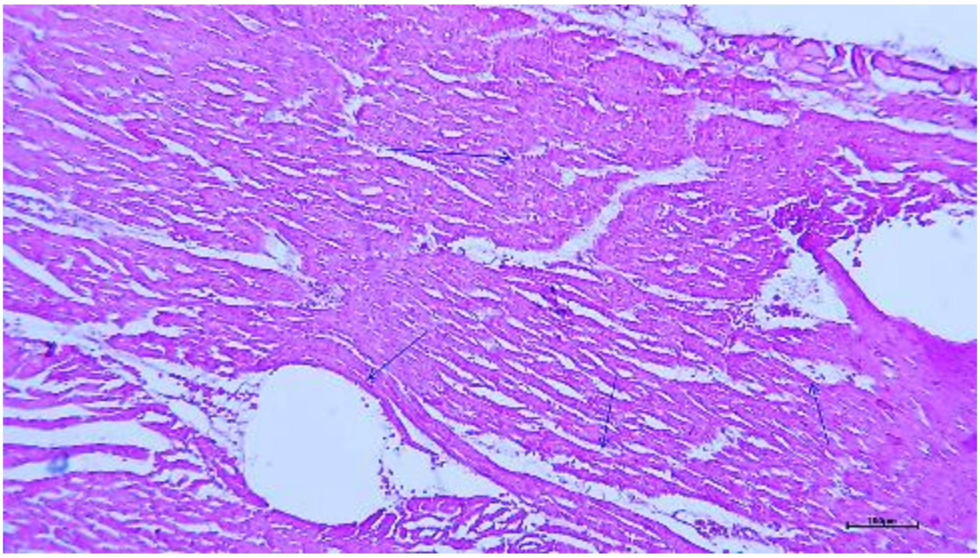

Fig. 10. Muscle degeneration, oedema and tearing, skeletal muscle, P.p., H&E, bar = 100 µm

The examined ovaries of P.p. showed clear signs of active ovarian cyclicity with presence mostly of primordial, secondary and few atretic follicles with scarring, as described by other authors. The uterus had typical feature of involuted uterus, in lamina propria of the endometrium, a lot of hemosiderin deposits were found.

Histologically the testes showed dense parenchyma with abundance of convoluted seminiferous tubules predominating over the rete testes. The tubules contained spermatids and spermatozoa. The examined *P.p.* testes had the feature of a mature active male.

Conclusions

The presence of adult animals with good nutritional status, stomachs with fresh or half-digested content, ubiquitous hyperemia, microhemorrhages and accumulation of gas bubbles in tissues are considered specific (pathognomic) signs of changes characteristic for cetaceans entangled in fishing gear as bycatch. Observed changes are similar with those described in more detailed previous studies. High share of lung samples with pneumonia condition are well in line with previous findings made within BLASDOL project in the end of 20th century.

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