

Project Update: February 2022

LABORATORY STUDIES

Sharks, skates and rays caught as by-catch within trawls, longlines and driftnets and that were already dead on landing were sampled for further laboratory analysis.

Biomarkers for water pollution and diseases

Melanomacrophages (MMC) are considered to be very sensitive, albeit non-specific, health indicators in the environment and were used as biomarkers of water pollution. Collected tissue samples from spleen and liver were routinely processed and stained with hematoxylin-eosin for further MMC examinations. Morphometric analysis was based on the 10 random high-power fields (HPF, 400x) on each liver/spleen slide following the methodology from our previous studies across the eastern Adriatic (Gajić et al., 2020, 2021, 2022). The number of MMCs, average volume of MMC per μm^2 and percent area of HPF occupied with MMCs were measured with sophisticated imaging software in 24.000 μm^2 area. Between December 2021 and February 2022, we have sampled and analysed 16 elasmobranch species landed in Radhimë, Triport, or Plazhi i Vjetër Vlorë (six sharks and ten batoids, Fig- 1-4), as shown in the table.

Studied elasmobranch species between December 2021 and February 2022 and their IUCN status in the Mediterranean Sea according to the latest assessment

Species name and authority	English name	Status
<i>Hexanchus griseus</i> (Bonnaterre, 1788)	Bluntnose sixgill shark	LC
<i>Galeus melastomus</i> Rafinesque, 1810	Blackmouth catshark	LC
<i>Mustelus punctulatus</i> Risso, 1827	Blackspotted smoothhound	VU
<i>Scyliorhinus canicula</i> (Linnaeus, 1758)	Lesser-spotted catshark	LC
<i>Scyliorhinus duhameli</i> (Garman, 1913)	Duhamel's catshark	NE
<i>Scyliorhinus stellaris</i> (Linnaeus, 1758)	Nursehound	NT
<i>Raja</i> (=Malacoraja) <i>clavata</i> Linnaeus, 1758	Thornback ray	NT
<i>Raja miraletus</i> Linnaeus, 1758	Brown ray	LC
<i>Raja asterias</i> Delaroche, 1809	Starry skate	NT
<i>Dipturus oxyrinchus</i> (Linnaeus, 1758)	Longnosed skate	NT
<i>Myliobatis aquila</i> (Linnaeus, 1758)	Common eagle ray	VU
<i>Aetomylaeus bovinus</i> (Saint-Hilaire, 1817)	Bull ray	CR
<i>Pteroplatytrygon violacea</i> (Bonaparte, 1832)	Pelagic ray	LC
<i>Dasyatis pastinaca</i> (Linnaeus, 1758)	Common stingray	VU
<i>Torpedo</i> (Linnaeus, 1758)	Common torpedo	LC
<i>Torpedo marmorata</i> Risso, 1810	Marbled electric ray	LC

Samples of liver, brain, gallbladder, pancreas, spleen, kidney, gills, entire digestive system, thyroid gland, rectal gland, entire urogenital (male samples) and genital system (female samples) were collected for histopathology. Our preliminary data pointed out two tumours in the cardiac stomach of the adult bull ray, while in other species mostly mixed inflammatory aggregations and degenerative changes were observed. The results are to be published in the peer-reviewed journals.

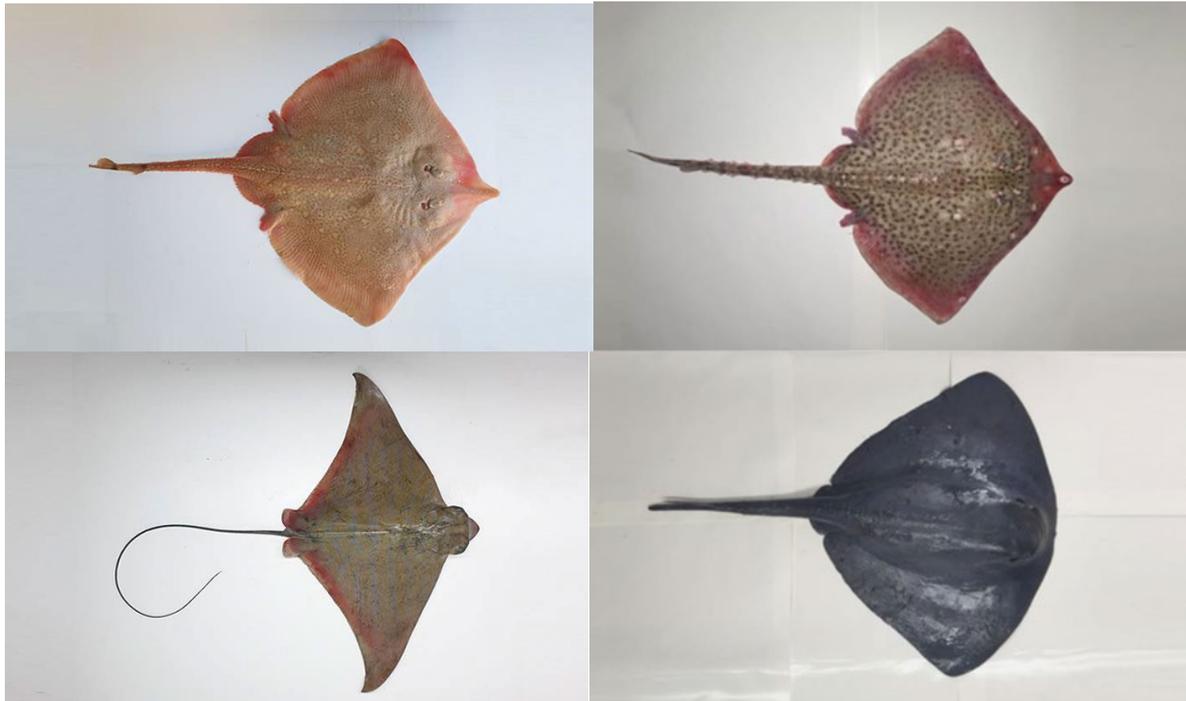


Fig. 1-4: Several commonly observed batoids in the Vlorë County: *Raja asterias* Delaroche, 1809 (up, left), *Raja* (=Malacoraja) *clavata* Linnaeus, 1758 (up, right), *Aetomylaeus bovinus* (Saint-Hilaire, 1817) (down, left) and *Pteroplatytrygon violacea* (Bonaparte, 1832) (down, right). © A. Gajić

Post-release survival rates

Individuals that showed any signs of life were immediately subjected to resuscitation in our oxygen-rich quarantine aquariums and were released back to the sea after 3 days of detailed stress measurements and observation. In such a way, we have released numerous gravid catsharks (*Scyliorhinus canicula* and *Scyliorhinus duhameli*), blackspotted smooth-hounds (*Mustelus punctulatus*) and marbled electric rays (*Torpedo marmorata*). Obtained scientific data will be used to increase the post-release survival rates.



Fig. 5-6: Gravid catsharks and torpedo rays caught by bottom trawlers and resuscitated in our oxygen-rich quarantine aquariums, prior to release. © A. Gajić.

About 25% of individuals died in the first 48 hours of the quarantine and were sampled for pathological studies. Autopsy and further histopathology revealed

significant trauma and internal bleeding in all dead animals, which resulted from the physical injuries upon the removing from the nets and staying on deck.

Bluntnose sixgill shark

Despite being a common and among the most abundant deep-sea sharks in the Mediterranean, the bluntnose sixgill shark is still considered rare in the eastern Adriatic Sea. There were no records during the deep-sea trawling studies through MEDITS (1994-2008) and FAO Adria Med (2008 – 2010). During the project, we have identified 31 new records in Albanian territorial waters (Fig. 7-8), caught in the outer shelves and upper slopes (100 and 600 m depth) by February 2022. Together with 10 other new records (eight in Croatia and two in Montenegro), they are described in the new paper that is currently under review. Condition factor was calculated for 25 of 42 specimens. Basic dental morphology and plausible feeding behaviour are further discussed in the paper.





Fig. 7-8: Adult female (500 cm TL, 410 kg TW) sampled in Triport, Vlorë. © A. Gajić.

Micro- and nano plastics

The digestive system of selected individuals was isolated for further qualitative and quantitative micro- and nano plastic studies. Basic steps from the adapted protocol were as follows. Upon the isolation, organs were rinsed in H_2O , measured, weighed, and transferred into separate Erlenmeyer flasks. The biological material was digested via dilution with filtration, wherein 20 ml of 30% H_2O_2 was added per 1 g of wet weight. Samples were incubated for 24h at 65 °C in a bain-marie, a type of heated bath. The suspension was filtered through the Macherey-Nagel cellulose filter papers with retention capacity of 1 to 2 μm - grades MN 640 de (thickness 200 μm , weight 100 g/m²) and MN 619 (thickness 170 μm , weight 75 g/m²). Upon the filtration, filter papers were placed into the petri dishes and left to dry for 24h. Plausible microplastics fibres, fragments, films, and pellets were identified using stereo microscope and secluded for Fourier-transform infrared (FTIR) and RAMAN spectroscopy.

List of researchers involved in the studies

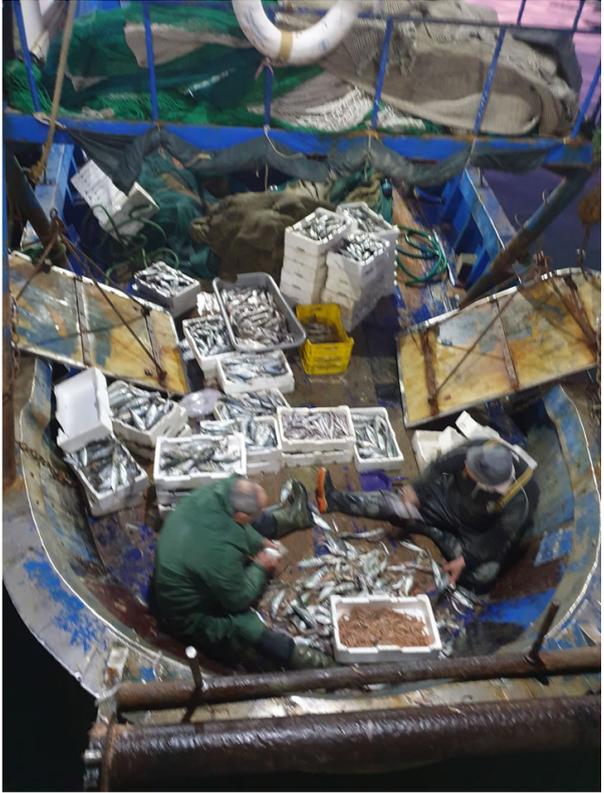
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Bottom trawlers in Triport January 2022.



Monitoring the landed sharks in Triport December 2021.



Hexanchus griseus and Andrej Gajic in Triport Jan 2021.