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Article

Predatory Responses and Feeding Behaviour of Three Elasmobranch Species in an Aquarium Setting

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Abstract: Many progressive aquariums worldwide house various elasmobranch species as part of their commitment to conservation awareness and the long-term well-being of these creatures. These aquariums face the challenge of enabling these natural predators to live harmoniously with other fish without triggering natural predation. This research, conducted at Zoomarine Algarve in Southern Portugal, aimed to investigate the behaviour of three elasmobranch species (*Carcharhinus melanopterus* (1:1:0), *Triaenodon obesus* (1:0:0), and *Pteroplatytrygon violacea* (0:3:0)) when exposed to different feeding mechanisms. The goal was to provide them with opportunities for alternative predatory behaviours beyond their typical feeding techniques and to reduce the likelihood of natural predation. The study took place under controlled conditions within a community habitat. Four feeding methods (pole, short buoy, long buoy, and PVC) were tested during morning, afternoon, and evening periods, using five different prey species. The results shed light on which feeding method aligns best with each species' distinct physiological standards and predatory tendencies and revealed their prey preferences. All three species interacted with all feeding methods, with *P. violacea* showing a strong preference for the pole method. *T. obesus* favoured bony fish, while *C. melanopterus* showed a preference for cephalopods. *P. violacea* interacted with all prey types but displayed no marked preference. These various feeding methods and prey options also function as environmental enrichment strategies, enhancing the complexity of the habitat and providing the animals with more choices and control, ultimately promoting their welfare in captivity.

Keywords: predatory responses; feeding behaviour; *Carcharhinus melanopterus*; *Triaenodon obesus*; *Pteroplatytrygon violacea*; Zoomarine aquarium

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1. Introduction

As apex predators, sharks play critical roles in the oceans by providing various benefits to the ecosystem. They are key figures in maintaining the balance of the ecosystem's trophic food web, and their removal can lead to disruptions in the trophic cascade [1,2]. The rapid decline of sharks and rays worldwide due to direct and indirect fishing activities has become a matter of great concern, posing a major threat to the sustainability of elasmobranch populations globally [1,3,4]. Unlike Teleosts, elasmobranchs (cartilaginous fish, such as chimaeras, sharks, and rays) share a similar life history pattern, characterised by slow growth, late sexual maturity and, consequently, late reproduction, and low fecundity. These factors contribute to their heightened sensitivity to external pressures, making them vulnerable to anthropogenic stressors and susceptible to population declines worldwide [5–7].

1.1. Integrated Welfare Approach in Aquariums

Today, the European Association of Zoos and Aquaria (EAZA) stands as one of the world's most politically influential and conservation-oriented institutions. One of its primary goals is to establish and maintain the highest standards of care and population management for the complete zoological collection of its members. To accomplish this, members are strongly encouraged to adhere to husbandry guidelines, specifically the "Standards for the Accommodation and Care of Animals in Zoos and Aquaria" [8], which is an essential tool for managing and ensuring the welfare of captive animals, including elasmobranchs. For instance, designing appropriate enclosures and considering the behavioural needs of each species promotes the expression of more natural behaviours and reduces unnatural behaviours. This is of particular importance since sharks and other apex predators in aquariums may exhibit predatory behaviour that can result in the natural predation of other inhabitants of the habitat [9,10] (personal observation). Given that animal welfare is closely linked to an understanding and promotion of animals' natural behaviours, it is highly recommended, according to the aforementioned guidelines, to encourage the expression of natural behaviour in zoos and aquariums [8]. Although there is a substantial amount of targeted research exploring the relationship between behavioural ecology, environmental complexity, and the overall welfare of captive animals, there is, to our best knowledge, a limited number of studies investigating the behaviour and welfare of fish species, especially those housed in zoos and aquariums [11]. This shortage is particularly evident in research that aims to analyse the natural behaviours of sharks beyond their typical diet and feeding routine, such as pole feeding, free diving, and unselective feeding.

1.2. Care Routine

For elasmobranchs, a healthy diet should be abundant in essential nutrients, such as proteins, carbohydrates, and fatty acids, which are readily available in the wild. However, in captivity, the use of supplemental vitamins and minerals has become a common practice in aquariums [12]. Following the EAZA's guidelines, aquarists typically collaborate closely with veterinarians to monitor the health status of each individual and pay meticulous attention to their daily routines. This includes, among other tasks, monitoring their feeding sessions, swimming behaviours, and physiological and ecological activities, such as territorial confrontations or predatory responses. Whenever feasible, sharks and other elasmobranchs are individually fed to ensure a more precise control of their diet.

1.3. Study Objectives

The primary objective of this study was to investigate the behaviour of three elasmobranch species, namely the Blacktip Reef Shark (*Carcharhinus melanopterus* (Quoy & Gaimard, 1824)), the Whitetip Reef Shark (*Triaenodon obesus* (Rüppell, 1837)), and the Pelagic Stingray (*Pteroplatytrygon violacea* (Bonaparte, 1832)), when exposed to various feeding mechanisms within a controlled environment in a community habitat at Zoomarine Algarve, Portugal. Our aim was to determine the most suitable feeding method for each species, considering their distinct physiological and ecological characteristics. We also identify the most effective way for these animals to feed on prey while reducing the risk of any natural predation and promoting natural behaviours. Through the expected knowledge gained, we hope to contribute to the ongoing improvement of welfare plans within the specific context of the aquarium under study. For this, two specific research questions guided this study:

1. Is there a specific time of day that is more favourable for these species to interact with the feeding methods?
2. What is the most appropriate feeding method for each species, taking into account their natural predatory tendencies (opportunistic vs. selective)?

2. Materials and Methods

2.1. Study Area and Sample

The study was conducted during September and October 2020 over a total of 30 sampling days at Zoomarine Algarve, an oceanographic park situated in southern Portugal that hosts a diverse range of species on display. Within the park, there is an aquarium, a significant zoological attraction, which consists of approximately 20 distinct themed aquariums and houses a wide variety of species. The largest central aquarium, containing around 400,000 L of seawater, serves as a community habitat and hosts numerous species, spanning tropical to temperate environments. Among the many species inhabiting this habitat, several elasmobranch species coexist. At the time of this research, the elasmobranch population included 2 *Carcharhinus melanopterus* (1:1:0), 1 *Triaenodon obesus* (1:0:0), 4 *Pteroplatytrygon violacea* (1:3:0), 3 Common Stingray (1:2:0) (*Dasyatis pastinaca* (Linnaeus, 1758)), 3 Common Eagle Rays (0:3:0) (*Myliobatis aquila* (Linnaeus, 1758)), 2 Common Guitarfish (1:1:0) (*Glaucostegus cemiculus* (Geoffroy Saint-Hilaire, 1817)), and 1 Undulate Ray (0:1:0) (*Raja undulata*, (Lacepède, 1802)). The selection of the three species for this study was made with the aim of reducing natural predation and encouraging other behaviours associated with animal welfare. One *P. violacea* (1 male) was excluded from the study because it displayed greater interest in reproduction than in food and was temporarily relocated to a secondary aquarium.

2.2. Species under Study

Given the ecological and physiological distinctions between these three species, they were deemed ideal candidates for gaining a better understanding of their feeding behaviours and for analysing their predatory responses. The three chosen species exhibit distinct predatory responses but share a broad diet range, encompassing cephalopods, crustaceans, and various species of teleost [13–15]. *C. melanopterus*, commonly known as the Blacktip Reef Shark, is a diurnal predator, typically found in coral reefs and shallow sand flats, and it can reach lengths of 1.0–1.07 m [16–18]. It functions as an apex predator and is known for its cooperative behaviour, often forming small groups to capture prey, and engaging in complex social interactions. They feed on a variety of small to medium-sized fish species, including reef fish, herring, mullet, and other small prey fish [19,20]. *T. obesus*, the Whitetip Reef Shark, is also a medium-sized shark, with a total length of approximately 1.8 m. Unlike the Blacktip Reef Shark, it is not an obligatory ram-ventilator, which means it can rest on the seabed or within caves. It is primarily considered a nocturnal predator, displaying increased predatory activity during night-time hours. It preys on reef fish, such as damselfish, parrotfish, wrasses, and other similar species, as well as cephalopods and benthic invertebrates, such as crabs, shrimp, and other small crustaceans [21–23]. *P. violacea*, the Pelagic Stingray, inhabits tropical, subtropical, and temperate environments and typically has an average disc width ranging from 44 to 47 cm [24]. It is recognised as a highly efficient diurnal epipelagic predator due to its effective use of its pectoral fins, feeding on cephalopods, small fish, including schooling fish such as herrings, anchovies, and other small baitfish [25]. When capturing prey, it wraps its fins around the target, drawing it closer to the seabed, thereby trapping and redirecting the prey into its mouth and preventing it from escaping [26].

2.3. Regular Feeding Routine

Daily feeding takes place in the afternoon, involving various types of prey ranging from cephalopods to bony fish. The feeding process for *P. violacea* occurs in two ways. Firstly, they can be fed during the general feeding session when aquarists disperse various types of food into the aquarium to feed all the inhabitants. Additionally, food is directly provided by aquarists during free dives, which are conducted three times a week. During these dives, aquarists also conceal elasmobranch tablets (which are supplemental vitamins) within the food that is administered directly to the intended individual. Shark species are fed using the pole method, whereby food is positioned at the end of a pole and presented to the shark as it swims past the pole. While all species have their nutritional requirements fulfilled through a diverse diet, this approach is still employed to more efficiently oversee their dietary needs, as frozen food may not provide all the essential nutrients and vitamins. From time to time, although very sporadically, the sharks may feed on small fish in the aquarium (usually tropical and temperate fish, i.e., butterfly fish and mullet fish), which are natural prey of the *T. obesus* and *C. melanopterus*, respectively. Although such predation events are very rare (less than 10 fish per year on record), aquarists have observed them typically occurring when the water temperature is unusually high or during periods of increased caloric demand, such as the mating season (personal observation).

2.4. Sampling Methods

To address the first research question, each day was structured with three experimental sessions (morning, afternoon, and evening) to observe the activity of each target species. Given that the aquarium is an enclosed facility, efforts were made to replicate natural lighting conditions throughout the circadian cycle. The timing and lighting conditions were set up as follows:

- Morning session: 8:30/9:00 am (lights off to simulate night-time conditions)
- Afternoon session: 2:30/3:00 pm (lights on to replicate daytime conditions)
- Evening session: 5:00/5:30 pm (lights off to recreate night-time conditions)

Each session had a duration of 30 min, during which all interactions of each subject were recorded. An interaction was defined as any behaviour indicating a noticeable interest in the presented feeding method. This could include attempting to feed, actual feeding, or displaying a general interest. Such behaviours might involve actively swimming towards the feeding structure, adjusting the swimming path towards the structure, circling the structure, or making physical contact with the structure, either with or without removing and ingesting the prey. Prey preference was assessed based on the successful ingestion of the prey. For a comprehensive description of behaviours specific to each species, please refer to Appendix A, Table A1.

To address the second research question, each session incorporated 1 or 2 (in the case of the buoy methods) out of 4 distinct feeding methods to assess the interest of each species. To prevent the subjects from becoming accustomed to a specific method and potentially learning from it, a different method was examined each day. The selection of methods was randomised throughout the study period to minimise any potential biases.

Five different prey species: European Flying Squid (*Todarodes sagittatus* (Lamarck, 1798)), European Squid (*Loligo vulgaris* (Lamarck, 1798)), Atlantic Herring (*Clupea harengus* (Linnaeus, 1758)), Atlantic Mackerel (*Scomber scombrus* (Linnaeus, 1758)), and Atlantic Horse Mackerel (*Trachurus trachurus* (Linnaeus, 1758)), which are consistently available year-round from regular suppliers, were tested across all feeding methods.

To provide the studied species with an opportunity to avoid the feeding frenzy often seen in community aquariums with larger fish, two distinct feeding locations were assigned: the primary feeding area (PFA), where aquarists typically feed all the fish in the central aquarium, and a secondary feeding area (SFA), located alongside the primary area within the central aquarium. The SFA is known to experience regular activity from the species under study. This arrangement allowed the subjects to explore the feeding methods without the disruption caused by other species in the primary feeding area.

2.5. Feeding Methods

In order to avoid the influence of confounding variables, such as other species simultaneously interacting with the methods, the design of the feeding methods took into account the predation habits of the extant species. Both pelagic methods were designed to have minimal impact on the remaining elasmobranchs, as they predominantly have benthic feeding habits. The PVC method, situated at the bottom of the aquarium, was the only one that could potentially attract other inhabitants. A preliminary experiment was conducted during the method testing, which confirmed that there was no significant interest from the other non-target species. Additionally, all other fish in the aquarium refrained from approaching the methods during the study, primarily due to the presence of the sharks and rays.

Pole Method: Method 1 involved a plastic pole measuring 135 cm in length. Each end of the pole was marked with three coloured insulation tapes (green, blue, and red), with each colour segment being 12 cm long. An airline tube was placed inside the pole, forming a loop at one end for grasping the prey, while the other end featured the airline tube tip, extending 100 cm and manipulated by the aquarist. The colours on the pole helped in adjusting the depth at which the prey should be handled. In this study, the prey was consistently placed at the blue tape. When an individual grabbed the prey, the airline tube was released (see Figure 1a,b). Two feeding poles were used in each session, with the prey suspended from one end of each pole, while the other end was held and manipulated by the staff member. Both poles were simultaneously immersed in the water. Every time a subject seized the prey, the cord was released to facilitate the removal of the prey. When the pole ran out of prey due to ingestion or falling into the aquarium, it was replaced until the end of the sampling period.

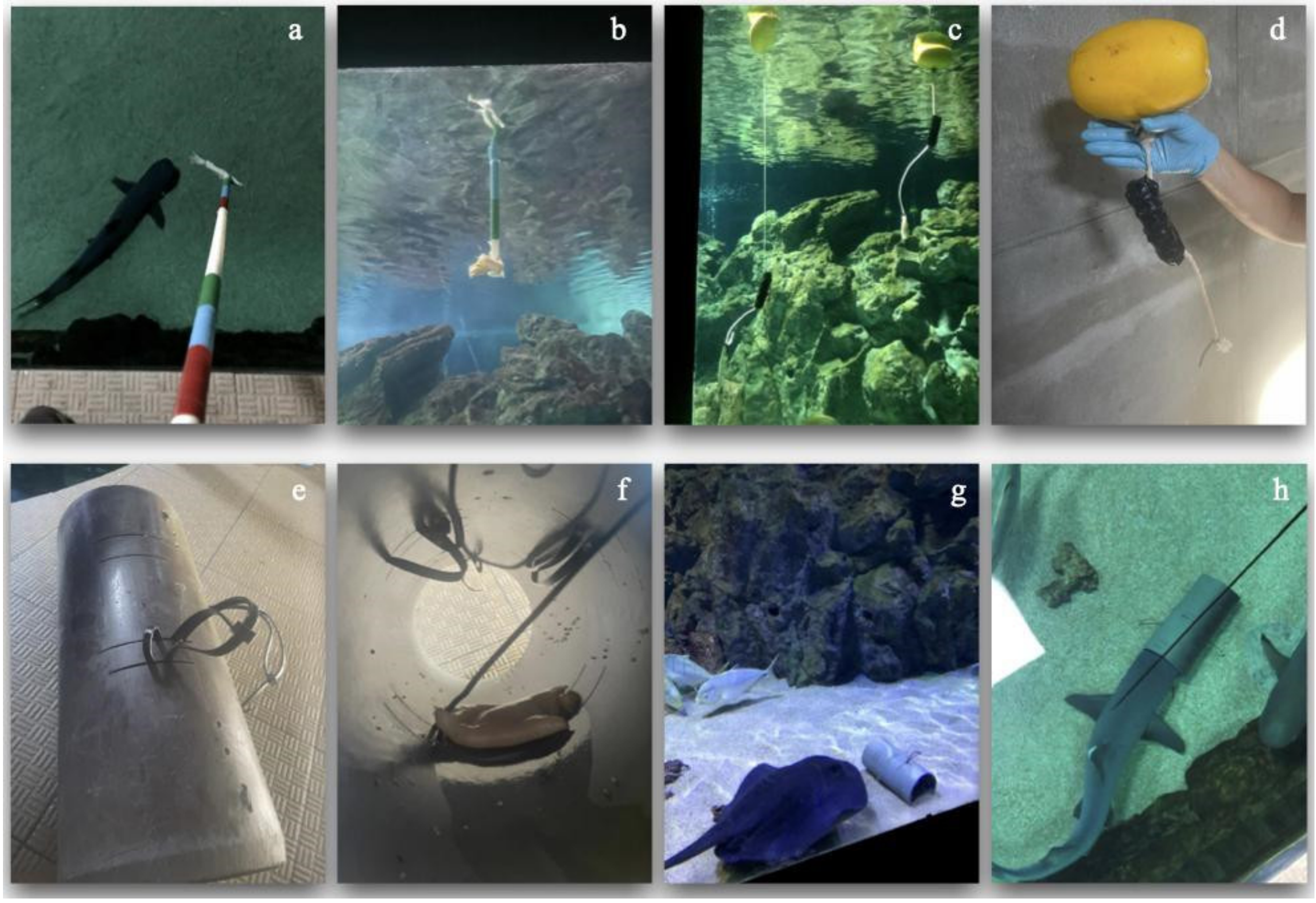

Figure 1. Visual examples of the methods under study. (**a**,**b**) The pole method, (**c**) both long (110 cm long) and short (40 cm long) buoys in the water, (**d**) details of the short buoy, (**e**) the PVC structure used in the PVC method, (**f**) inside of the PVC tube with food, and (**g**,**h**) the deployment of the PVC tube method.

Buoy Method: The buoy method involved the use of two buoys, each of different lengths (short and long), positioned in the water column with the prey suspended at varying depths. Each buoy was composed of a rope, a buoy at the top, a diving weight positioned in the middle of the rope, and a plastic clamp at the bottom to attach the prey. The short buoy was constructed with a 40 cm rope, while the long buoy had a 110 cm rope. Each feeding area featured a pair of buoys (long and short), and all buoys were simultaneously placed in the water column (see Figure 1c,d). The behaviour of the target species was recorded whenever they interacted with the method. As each buoy depleted its prey, the prey was replaced until the end of the sampling period. The use of different lengths aimed to assess species preferences.

PVC Tube Method: The PVC method involved two PVC tubes placed in the substrate or at the bottom of the tank. Each PVC tube measured 45 cm in length and had a diameter of 20 cm, with side cuts used to attach three plastic clamps. Two of these clamps were used to secure the prey inside the tube, while one was used to assist in retrieving and removing the tube from the water using a hook (see Figure 1e,h). This method kept the prey contained within the tubes during the feeding process. Both tubes were released into the water column simultaneously, and the behaviours of the animals were observed. Each time a tube ran out of prey, the prey was replaced until the end of the sampling period.

2.6. Data Analysis

All data were analysed using IBM SPSS Statistics, Version 23. Friedman tests were used to compare the interactions of each species between the three periods (morning, afternoon, and evening). Interactions were recorded as continuous variables. The Shapiro–Wilk test was first performed to test for normal distribution. Descriptive data were compared using Friedman's tests, while Wilcoxon's signed rank tests were used for post hoc testing. To facilitate the interpretation of the results, data were merged by species, not individuals. Significant differences were considered for $p < 0.05$.

3. Results

3.1. Preliminary Analysis

Normal distribution was first analysed. For this, a Shapiro–Wilk test of normality was performed between the two feeding areas (PFA and SFA) for each species, independent of the period or feeding method. Since the data did not follow normal distribution for this larger sample, subsequent analysis followed a non-parametric approach. A Friedman test was then used to determine differences between feeding areas. Since no differences were found between areas for any of the species under study (*T. obesus* (X^2 (1) = 0.111; $p = 0.74$); *C. melanopterus* (X^2 (1) = 0.04; $p = 0.84$); *P. violacea* (X^2 (1) = 3.60; $p = 0.06$)), the two areas were merged into one single sample in order to increase sampling reliability.

3.2. Preferred Daily Period

Table 1 shows the mean and standard deviation values of the interactions reported for each sampling period of the day per species under study.

Table 1. Descriptive statistics of the reported interactions for each period of the day for the species under study. Mean (SD) and median (IQR) values for each period of the day across the 30-day sampling period.

	T. obesus		***C. melanopterus***		***P. violacea***	
Period	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**
Morning	1.70 (2.20)	1.00 (0.00–3.00)	0.54 (1.17)	0.00 (0.00–1.00)	6.35 (4.71)	6.00 (3.00–7.75)
Afternoon	0.44 (0.78)	0.00 (0.00–1.00)	0.46 (1.37)	0.00 (0.00–0.00)	7.08 (5.52)	6.00 (3.00–10.00)
Evening	0.83 (1.47)	0.00 (0.00–1.00)	0.85 (1.96)	0.00 (0.00–1.00)	5.91 (5.17)	5.00 (2.00–8.75)

All but one species (*T. obesus*) showed no significant differences between periods. *T. obesus* showed significant differences between periods (X^2 (2) = 18.17; $p < 0.001$). Post hoc comparison for *T. obesus* suggested that all periods significantly differed from each other (morning vs. afternoon: $Z = 4.48$; $p < 0.001$; morning vs. evening: $Z = 2.86$; $p < 0.01$; afternoon vs. evening: $Z = 2.20$; $p = 0.03$). To decrease the number of conditions under study and further simplify the analysis, the morning period was chosen as the most representative period of the day for all species. Therefore, from this point onwards, our interpretation refers only to the morning period.

3.3. Feeding Method Preference

Table 2 shows the preference scores of each species for each of the feeding methods under study.

Table 2. Descriptive statistics of the reported interactions with each feeding method for the species under study. Mean (SD) and median (IQR) values for the morning period across the 30 sampling days.

	T. obesus		***C. melanopterus***		***P. violacea***	
Method	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**
Pole	2.20 (2.55)	1.00 (0.00–4.00)	0.20 (0.41)	0.00 (0.00–0.00)	9.80 (5.91)	7.00 (6.25–14.50)
Long buoy	1.30 (1.81)	0.50 (0.00–2.75)	0.55 (1.23)	0.00 (0.00–0.75)	6.95 (4.20)	6.50 (3.50–8.75)
Short buoy	1.45 (2.01)	0.50 (0.00–2.75)	0.55 (1.32)	0.00 (0.00–0.00)	5.15 (2.56)	5.50 (3.25–6.00)
PVC	1.85 (2.41)	1.50 (0.00–2.00)	0.85 (1.42)	0.00 (0.00–1.00)	3.50 (3.22}	3.00 (1.00–5.75)

No differences were found between methods in both shark species (*T. obesus*: (X^2 (2) = 1.05; $p = 0.79$); *C. melanopterus*: (X^2 (2) = 2.61; $p = 0.46$)). *P. violacea* showed differences between methods (X^2 (2) = 17.62; $p = 0.001$). A detailed comparison for *P. violacea* showed differences between the pole and the short buoy methods ($Z = 2.81$; $p = 0.005$), the PVC tube and pole methods ($Z = 3.61$; $p < 0.001$), and the PVC tube and the long buoy methods ($Z = 2.18$; $p = 0.03$).

A detailed analysis of the prey preference of each shark species showed a clear and distinct prey preference (Table 3).

Table 3. Descriptive statistics for prey preference for each species under study. Mean (SD) and median (IQR) values for the morning period across the 30 sampling days, independent of the method used.

	T. obesus		***C. melanopterus***		***P. violacea***	
Prey	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**
Atl. Mackerel	0.94 (1.83)	0.00 (0.00–2.00)	0.17 (0.52)	0.00 (0.00–0.00)	9.06 (7.00)	7.50 (3.25–12.75)
Eur. Squid	0.44 (1.05)	0.00 (0.00–0.00)	0.96 (2.02)	0.00 (0.00–0.75)	6.85 (4.69)	6.50 (4.00–9.75)
Eur. Flying Squid	0.92 (1.37)	0.00 (0.00–2.00)	1.15 (1.58)	0.00 (0.00–2.00)	5.65 (5.21)	4.00 (2.00–7.00)
Atl. Herring	1.15 (1.85)	0.00 (0.00–2.00)	0.31 (0.70)	0.00 (0.00–0.00)	6.56 (4.58)	6.00 (3.25–9.00)
Atl. Horse Mackerel	1.50 (1.97)	0.50 (0.00–2.75)	0.12 (0.49)	0.00 (0.00–0.00)	5.13 (4.19)	4.50 (2.00–7.00)

All species showed marked preferences for specific prey (*T. obesus*: (X^2 (4) = 9.88; $p = 0.04$); *C. melanopterus*: (X^2 (4) = 31.31; $p < 0.001$); *P. violacea* (X^2 (4) = 13.56; $p < 0.01$). The European Squid was markedly the least preferred prey for *T. obesus*. This species showed a noticeable and significant preference for the Atlantic Herring ($Z = 2.16$; $p = 0.03$), Atlantic Horse Mackerel ($Z = 2.93$; $p = 0.003$), and European Flying Squid ($Z = 2.02$; $p = 0.04$) when compared to the European Squid. In contrast, *C. melanopterus* showed a clear preference for cephalopods when compared to teleosts. Significant differences were found between the European Flying Squid and the Atlantic Herring ($Z = 3.42$; $p = 0.001$), Atlantic Mackerel ($Z = 3.46$; $p = 0.001$), and the Atlantic Horse Mackerel ($Z = 3.70$; $p < 0.001$). The European Squid showed significant differences when compared to both the Atlantic Mackerel ($Z = 2.91$; $p = 0.004$) and the Atlantic Horse Mackerel ($Z = 2.58$; $p < 0.01$). On the other hand, *C. melanopterus* did not exhibit a marked preference among cephalopod species as its preferred prey. *P. violacea* exhibited a higher level of interest in the Atlantic Mackerel, as evidenced by its average values (Table 3). The Atlantic Horse Mackerel was its least preferred prey, as shown by the statistical differences found between the European Flying Squid ($Z = 3.00$; $p = 0.003$), the European Squid ($Z = 2.86$; $p = 0.02$), and the Atlantic Mackerel ($Z = 2.90$; $p = 0.004$).

4. Discussion

This study aimed at testing different feeding mechanisms for three different elasmobranch species under controlled conditions. We were able to determine the most favourable period of the day for these species to interact with the feeding methods. We further defined the most appropriate feeding method, if any, for each species, considering their different biological and ecological characteristics.

4.1. Preferred Period of the Day

T. obesus are known to rest during the day on reef ledges or in caves and are more active during the night-time when they come out and feed [22,23,27]. Our results are coherent with this, as *T. obesus* was the only species to show a preference for the morning sampling period, which mimicked night-time conditions. The other two ram-ventilator species showed no preference for the period of the day to feed, which was expected due to their constant need to swim in order to breathe. This leaves them with a continual expenditure of energy, forcing them to constantly forage for food, independent of the period of the day [28].

4.2. Preferred Feeding Method

By testing different feeding methods, we aimed at understanding how the natural predatory habits of each species could be influential in engaging their interest and, thus, be included as an enrichment strategy. While no differences were found between methods for both shark species, they did interact with all methods differently, highlighting the specific adaptations of each species. *P. violacea* showed a marked preference for the pole and long buoy methods, while showing constant interest, even though not successful in feeding, in other methods.

4.2.1. *T. obesus*

Our results show that *T. obesus* clearly preferred bony fish, namely Atlantic Horse Mackerel. *C. melanopterus* preferred cephalopods, such as the European Flying Squid, which is already part of the main diet for this species in the wild [17]. Even though *T. obesus* showed no preference for any particular method, it showed a more inquisitive behaviour when compared to *C. melanopterus*, something possibly explained by its own ecological foraging strategy, exploring the surrounding environment more frequently. *T. obesus* exhibit an individualised, inquisitive, and sedentary response to food [29]. It was, in fact, able to successfully capture prey with all methods, with a special remark for the PVC method, where it was able to enter the tube, grab the prey, and successfully get out of the tube by swimming in reverse. This behaviour and efficacy are probably related to their known capacity to use their electrosensory system to detect prey and scavenge for food between corals and rock holes [15], giving them an advantage to find and capture prey in narrow and unreachable spaces for other sharks. This species also showed more interactions with the pole method than we were initially expecting. Known to have a more cryptic behaviour, *T. obesus* showed no problem exploring the water column, competing to access the prey.

4.2.2. *C. melanopterus*

Blacktips are generally introverted predators that tend to hunt in groups, displaying increased excitement when other members of the same species are present during prey encounters [29]. This behaviour was observed during the study, with one individual's interest in prey often sparking immediate interest and excitement in another. In contrast to *T. obesus*, this species exhibited caution when encountering new objects (personal observation). Given the species' introverted nature, an extension of this research might reveal a rise in self-assurance, potentially resulting in heightened engagement with the feeding methods. Moreover, even though this species took a longer time to engage, once the methods were introduced in the water, they adopted a more foraging-oriented stance, demonstrating active food-seeking behaviour through increased swimming activity. No particular method was preferred by *C. melanopterus*, although the use of the PVC method needs a special remark. This species is known to engage in social foraging behaviour, where groups of predators work together to search, pursue, and capture prey [29]. This was observed more during the

PVC method, where both *C. melanopterus* circled the PVC tube, demonstrating interest, but in no situation did they attempt to remove the prey from the tube, as in trying to solve the "PVC puzzle" together. *C. melanopterus* share the same reef grounds as *T. obesus* but they usually feed in shallow water. Their torpedo-shaped bodies allow them to catch high-speed prey in near-coastal waters, while *T. obesus* have a more food-seeking behaviour while capturing prey [2]. A recent study showed evidence of heterospecific foraging associations between *T. obesus* and grey reef sharks (*Carcharhinus amblyrhynchos*) [29]. While *T. obesus* can reach hidden prey between the corals due to their high flexibility, the grey reef sharks patiently wait for the opportunity to steal the prey, giving them access to prey that would be inaccessible if they were hunting by themselves [29]. A similar foraging behaviour was found between the two shark species in this study.

4.2.3. *P. violacea*

P. violacea feed on the water column and they use their pectoral fins to handle and direct prey towards their mouths [30]. They showed higher interactions with the methods more adapted to their flat-shaped and highly mobile anatomical characteristics (the pole and buoy methods), where the prey was clearly exposed in the water column. Being the opportunistic forager par excellence within the three species [31,32], it is not surprising that the PVC method was the least effective for catching prey due to their body shape. Despite this, they showed a high interest in getting the prey from the PVC tube, actively swimming around the structure and, whenever the prey was more exposed or outside the PVC structure, they did try to catch it.

Even though all target species share a similar wide range diet in the wild, we also found differences in the prey preferences in this study. *P. violacea* demonstrated a noticeable lesser preference for the Atlantic Horse Mackerel, although, in general, displayed high interest in all available prey. This is understandable as they are considered opportunistic feeders; that is, they have a much more varied diet compared to the other two shark species, which predominantly have a prey type [31].

4.3. *Overall Considerations and Applications*

The two pelagic feeding methods (pole method and buoy method(s)) were particularly effective for *P. violacea* and should be implemented as additional enrichment strategies rather than as part of general feeding aquarium sessions. Despite the PVC method being somewhat ineffective for this species, the evident interest in it suggests that this method should still be considered as an extra strategy, provided the species' positive response to it is maintained. This will, however, require the inclusion of a consistent behavioural reporting effort in welfare routines. Concerning the two shark species, even though they did not exhibit any specific preference, special attention should be given to the PVC method. As both species displayed interest in solving the "PVC puzzle", our results indicate the need to include this method as another environmental enrichment strategy, fostering heterospecific relations between shark species. This will contribute to increasing environmental complexity and enhancing animal welfare in captivity.

It is important to acknowledge that, whether in aquariums or in the wild, animals tend to manage their energy costs wisely. In controlled environmental conditions, animals do not need to expend as much energy on foraging or hunting as they would in the wild, allowing them to allocate more energy to reproduction, rest, or growth [33]. This study also aimed to encourage natural predation behaviours through alternative methods. Instead of the animals expending energy on natural predation or traditional feeding methods (such as prey hanging on a pole), they spend their energy towards environmental enrichment methods, as presented here.

All in all, recognising prey preference in species in controlled environments provides an opportunity to use prey as a source of animal welfare strategies. Prey preference analysis can assist in making management decisions and performing essential tasks related to animal welfare, such as administering medication more easily by hiding it in the preferred food rather than undesirable food. The use of different feeding methods and prey availability increases complexity within the environment, offering animals more variety, choice, and control over their surroundings [34]. This has been proven to be linked to achieving good animal welfare, serving as an environmental enrichment strategy [35]. From a zoological management perspective, the use of environmental enrichment methods increases behavioural diversity and helps animals focus more on behaviours such as foraging rather than competition or natural predation [35,36]. To the best of our knowledge, there are still limited studies on the environmental enrichment of elasmobranchs or even studies related to fish behaviour and fish welfare in captivity that can contribute to improving research for evidence-based management decisions [35,37].

5. Conclusions

Studying the behaviour of aquarium species under diverse yet controlled conditions provides aquarists and other animal welfare managers with valuable insights into the specific behaviours of each species. This knowledge enables them to enhance the welfare of these species accordingly. The significance of such studies is particularly pronounced when examining species that inhabit community habitats, where constant interactions with other species occur. Our comprehensive findings indicate that all target species adeptly captured and selected prey across various methods and prey species.

Incorporating diverse feeding methods into the welfare plans for these selected species not only ensures their successful prey capture but also introduces the possibility of tailoring prey preferences for each species. This approach contributes to creating a more enriching environment for their daily routines. By delving into the behaviour of these aquarium species under various controlled conditions, we not only enhanced our understanding of their specific needs but also allowed for an evidence-based approach to their management, allowing the improvement of the aquarium's welfare plans and ensuring the well-being of the species under human care.

Limitations and Future Studies

This study has some limitations that deserve attention for future research. One prominent limitation was the sample size, both in terms of the number of individuals sampled and the duration of the study. The elasmobranch collection in the aquarium during this study was relatively small, significantly restricting the generalisability of our results to a broader context. As such, our results cannot be universally applied due to the study's small sample size and the unique ecological features of the habitat. Despite this limitation, it provided valuable insights into the importance of studying the individual behaviours of these long-lived species when exposed to various feeding strategies, facilitating the implementation of species-specific welfare practices in the host facility's welfare plan. Another limitation pertains to the study's duration, which lasted only 30 sampling days. Extending the sampling period could enhance the robustness of the results, particularly regarding the initially shy behaviour of *C. melanopterus*.

Additionally, a limitation is associated with the experimental design and the inherent characteristics of a controlled environment. Since all tested feeding methods were food-related, conducting a thorough year-round baseline analysis of the physiological parameters of each species would be crucial for a better understanding of energy-related investments when deploying these feeding methods.

Future studies on heterospecific relationships among reef sharks should be explored both in controlled conditions and in the wild to comprehensively understand trophic relationships among individuals sharing the same environment. Another prospective avenue for enrichment involves investigating the use of the elasmobranchs' electrosensory system for prey capture. All sharks and rays share this system, using it to locate prey. The electrosensory system can be categorised as active or passive, depending on the species' ability to actively produce a bioelectric signal or detect electric fields from external sources. While all elasmobranchs rely on passive systems, variations in morphology and density are related to their feeding habits. Developing enrichment strategies using electric stimuli with varying intensities and locations could provide additional options for each species. Furthermore, we strongly recommend studying the feeding mechanisms and predatory responses of elasmobranchs using an environmental enrichment approach, such as the ABA experimental design (baseline, enrichment, and return to baseline), to help define future enrichment strategies.

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Appendix A

Table A1. Examples of possible interactions of the species under study within each method.

Species under Study	Examples of Interactions	
T. obesus	Pole/Buoys	Swimming in close proximity to the method (within <1 m), altering the swimming path towards the method, circling the method, and touching the method with/without removing and/or ingesting the prey.
	PVC	Entering/exiting the method, swimming in close proximity to the method (within <1 m), altering the swimming path towards the method, circling the method, and touching the method with/without removing and/or ingesting the prey.
C. melanopterus	Pole/Buoys	Swimming in close proximity to the method (within <1 m), altering the swimming path towards the method, circling the method, and touching the method with/without removing and/or ingesting the prey.
	PVC	Swimming in close proximity to the method (within <1 m), altering the swimming path towards the method, and circling the method.
P. violacea	Pole/Buoys	Swimming in close proximity to the method (within <1 m), altering the swimming path towards the method, embracing the method, and touching the method with/without removing and/or ingesting the prey.
	PVC	Swimming in close proximity to the method (within <1 m), altering the swimming path towards the method, and standing stationary on top of or near the method.

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