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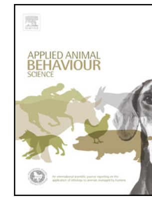

# Environmental enrichment in captive juvenile thornback rays, *Raja clavata* (Linnaeus 1758)

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ABSTRACT

There are few studies investigating captive conditions for commonly kept public aquaria species. Here the thornback ray (*Raja clavata*) was used to determine preferred captive conditions via choice tests and behavioural observations. Substrate type, substrate colour, substrate depth, group size and refuge use were all used to assess usage, number of stereotypic behaviours and activity in captive born rays. Sand was the preferred choice of substrate which also brought fewer surface breaking behaviours (a possible stereotypic behaviour) compared to gravel or bare tanks. Lighter colours of sand were preferred, as were deeper depths whilst increasing group size increased possible stereotypic behaviours. Type of resting behaviour (horizontal vs vertical) also differed within experiments – rays switched from horizontal to vertical resting, on the side of the tank when using gravelled versus sandy areas of the tank. The rays in this study appeared not to use refuges. Very few published studies have focused on what aquatic animals want, here we use preference tests, which are a useful way of determining what the animal wants, and can help aquarists provide the best conditions for captive thornback rays.



## 1. Introduction

### 1.1. Background

Elasmobranchs (i.e. sharks and rays) are commonly kept in public aquaria throughout the world. Despite being widespread, few published studies have investigated their wellbeing in captivity. The definition of wellbeing is debatable with most definitions falling into three different approaches; feelings-based, functions-based and nature-based, (Huntingford et al., 2006). Some authors, adopting a more feelings-based approach, such as Duncan and Fraser (1997) who suggested that, once animal health has been accounted for, enabling animals to perform desired behaviours (i.e. what animals choose) is important for their welfare. One way to quantify this, is with preference tests, where the animal is given a choice between different options.

The occurrence of abnormal stereotypical behaviours is commonly used to indicate stress in captive animals and are defined as repeated sequences of abnormal behaviours which appear to be functionless and have no apparent benefit to the animal (Dawkins, 1988; Mason et al., 2007). It is suggested that stereotypies may result when the animal is faced with an environment it has not evolved to cope with (e.g. restricted enclosure space), prompting a particular behavioural response, and induces constant stress affecting brain function or delayed central nervous system development during infancy (Mason et al., 2007; Clubb and Mason, 2007). Surface breaking behaviour (SBB), bobbing (BOB) and spiralling (SP) are stereotypical behaviours which are frequently observed in captive elasmobranchs (see Table 1) and may be a behaviour associated with captive feeding regimes (Casamitjana, 2004): the behaviours can be reduced with changes in the delivery of food items (Scott et al., 1999) i.e. providing food on the bottom of the tank as opposed to floating on the surface. However, SBB/BOB does not always occur close to feeding times with rays (Smith, 2006; Greenway pers. observation) suggesting the behaviour may have become stereotypic.

Increasing enclosure complexity with environmental enrichment may encourage a natural context which can encourage more foraging behaviours (Näslund and Johnsson, 2014; Kuppert, 2013). Uses of enrichment differs according to species, age, sex, reproductive state and ideally take into account the needs of the individual animal rather than a one size fits all approach used across a population or range of species (Corcoran, 2015). Thornback rays (*Raja clavata*) natural environment can be highly complex and some features can be artificially simulated making enclosures more natural and stimulating, for example; adding or changing water currents, (Kuppert, 2013; Naslund and Johnsson, 2014), creating refugia from natural or artificial materials, and creating foraging opportunities, all of which may increase stimulation and therefore reduce stereotypical behaviours (Naslund and Johnsson, 2014;

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**Table 1**
Ethogram of behaviours observed during the pilot study and experiments, descriptions of the behaviours included and stereotypical behaviours defined by Casamitjana (2004) and observed by the authors.

| Behaviour | Description | Definition |
|---|---|---|
| Resting | Horizontal Resting | Ventral side touching bottom of the tank, no or very little movement |
| | Burying | Oscillating outer body rapidly to move sand onto body |
| | Moving tail | Moving tail side to side while keeping rest of the body still. |
| Locomotory | Crawling | Ventrally moving using pelvic fins to push sand along the bottom of the tank |
| | Swimming | Moving through the water without touching the water surface or floor of the tank |
| | Jetting | When an individual quickly propels itself to move away from a stimulus |
| | Digging | Using snout and fins to move substrate |
| | Shake | Quickly moving body (leading with the head/snout) side to side. This can be done whilst vertical, horizontal or during a swimming behaviour |
| Thigmotaxis | Vertical Resting | Resting ventral side against the wall of the tank, no or very little movement |
| | Vertical Swimming | Swimming whist ventrally touching the wall of the tank |
| | Vertical Burying | Oscillating outer body whilst ventral side is against the wall of the tank |
| | Bopping | When resting vertically, the individual repeatedly moves up and down the side of the tank |
| Stereotypical Behaviour | Surface Breaking Behaviour | Swimming with head/snout above the water's surface* |
| | Spiralling | Swimming around a central point horizontally and vertically in a back flip motion[a] |

[a] denotes Casamitjana (2004).

Smith, 2006). The thornback ray is an epibenthic elasmobranch species commonly seen in public aquaria across the world (Smith, 2006) and in the wild spend most of their time partially buried in substrates (Dehart, 2004; Nottage and Perkins, 1983) and their environment is somewhat replicated in captive conditions. Many elasmobranchs are able to slowly match their colouration to the substrate through changes in their epidermal chromatophore cells (Sugimoto, 2002; Kemp, 1999), and may use camouflage to ambush prey (Wearmouth et al., 2014).

The frequently seen SBB and BOB stereotypy is unlikely to be natural given the rays natural history (i.e. significant anatomical adaptation to benthic living) and behavioural ecology (ambush predator or benthic scavenger (Wearmouth et al., 2014)). Surface breaking may even be unwittingly encouraged by aquarists as it allows the animals to be seen, and in some collections touched (i.e. via touch tanks), by the public. Although nearly always provided with a substrate, preference for any of the numerous types of substrate has never been tested.

Based on a study by Fowler (2005) the thornback ray is classified as near threatened on the ICUN database, due to slow growth rates, late maturity (Gallagher et al., 2005) and low fecundity they are highly vulnerable to trawl fisheries. This has led to declines in the global population (Chevolot et al., 2006; Smith and O'Connell, 2014). This conservation risk mean that successful captive breeding programmes may become essential and many captive organisms may not breed in captivity if welfare is poor (Mason et al., 2007).

### 1.2. Aims of study

Aquarium welfare is understudied compared to captive terrestrial animals and there are very few captive elasmobranch studies focusing on their welfare and environmental enrichment (Naslund and Johnsson, 2014), which reflects the paucity of aquarium welfare studies as a whole. The aim of this study is to identify preferences in a range of conditions which are consistent with common or plausible captive conditions (substrate, group size and refuges) via behaviour based experimentation. Ecological studies based on survey data are inconclusive over substrate preferences, with some studies suggesting thornback rays use sand and gravel equally (Maxwell et al., 2009), others suggesting juvenile rays may prefer sand over gravel (Martin et al., 2012), and some evidence to suggest that historically, in the North Sea, rays may have preferred sandy habitats but have moved into areas containing more gravel as these are lower-risk from fisheries (Fock, 2014). Therefore, there is a need to better understand substrate preferences of this species. Since rays are benthic, we predict they will avoid areas of bare tank. Substrates can harbour pathogens, promote anoxic areas and are difficult to clean, we investigated whether rays would accept a very shallow substrate which may mitigate against such issues.

Elasmobranchs are able to change morphological features in chromophores and respond to environments with varying light intensities (Sugimoto, 2002; Kemp, 1999) which are affected by environmental properties such as substrate colour, finding any preferences within their natural environment may better their psychological well-being and improve their welfare.

Public aquariums often keep rays in groups, often at a relatively high stocking density, in large shallow "ray tanks" (some aquariums may even allow the public to touch the animals). Not much is known about *Raja* spp. social behaviour (they group for mating and it is suggested outside of this the sexes are segregated (Nottage and Perkins, 1983), therefore it is important to understand the impact of increasing stocking density on individual ray behaviour. We therefore aim to investigate whether increasing the group size of individuals in a tank increases incidence of behaviours that may indicate stress.

Many animals in captivity benefit from the use of refugia (Näslund and Johnsson, 2014) and are also observed in some ray tanks in public aquaria. Uses for refugia in ray are yet to be tested and we observed for preferences between an 'artificial' (which might be used in stock/off show tanks e.g. for captive breeding programmes) or a 'natural' refuge (more likely to be used in display tanks, where typically a naturalistic effect is favoured by the public).

## 2. Materials and methods

### 2.1. Animal husbandry and facilities

14 juvenile rays (full siblings, 13 months old at the beginning of this study) were obtained from a public aquarium where they were hatched, all individuals were transported using the sealed bag and insulated box technique (Smith et al., 2004). Rays were kept in two stock tanks $1.46 \times 1.01 \times 0.35$ m (lxwxh) containing 5 individuals and $2.41 \times 1.06 \times 0.71$ m containing 9. Rays ranged in size from 15 cm (fin to fin) to 32 cm throughout the 8-month study period. Yellow sand was used as a substrate and no other furnishings were present. Tanks were cleaned by picking up excess food when necessary; water was replaced via a semi-open recirculating system within Bangor University's aquaria facility, and kept at seasonal ambient temperature ranging from 10 to 17 °C during the study period (October 2014–June 2015). Each experiment was performed in a short time period reducing confounding effect of temperature rising although this may have affected activity levels between experiments. Lights were on timers and turned on at 0700 and off at 1900. The rays were fed once ad libitum each day after the