



Original Research Paper

## Use of Ethograms to analyse Shoaling Behaviour of Mackerel (*Scomber scombrus*) in an Aquarium

Thomsen H<sup>1</sup>, Jensen TH<sup>1,2</sup>, Ydesen K<sup>3</sup>, Madsen N<sup>1</sup>

<sup>1</sup>Department of Chemistry and Bioscience, Aalborg University, Aalborg DK-9000, Denmark;

<sup>2</sup>Department of Zoology, Aalborg Zoo, Aalborg DK-9000, Denmark;

<sup>3</sup>North Sea Oceanarium, Hirtshals DK-9850, Denmark;

**Corresponding author:** Helena Thomsen, Department of Chemistry and Bioscience, Aalborg University, Aalborg DK-9000, Denmark; **Email:** [hthb11@hotmail.com](mailto:hthb11@hotmail.com)

### Abstract

The aim of this study was to design an ethogram to investigate mackerel (*Scomber scombrus*) shoaling behaviour in an aquarium. We divided shoaling behaviour into four categories: area of the tank where the shoal was present (position); the morphological shape of the shoal (formation); shoaling movements (movement) and the average distance between the individuals in the shoal (density). The shoal was observed in a non-feeding trial and a feeding trial where the shoal was fed with food pellets additional to the normal feeding schedule. The study was conducted in an aquarium with 4.5 million liters of water at the North Sea Oceanarium, Hirtshals, Denmark. Our results indicate that different patterns in the shoaling behaviour could be identified using an ethogram. The plate, which is a closed elliptical shape, was the most observed formation, while the forward unit, which is the average forward motion of the shoal moving horizontally, was the most observed movement. Light intensity, pH, oxygen level, and temperature showed no variation between the trials and thus does not explain any behavioural differences. The shoal showed no clear long-term reaction to feeding, though the shoal showed a preference for the top area when fed more regularly. Further investigations in shoal behaviour could determine whether these categories in behaviour could monitor stress level in confined shoals.

**Keywords:** aquarium; ethogram; shoal behaviour; mackerel; *Scomber scombrus*

### Introduction

Safety in numbers is a reoccurring phenomenon across the animal kingdom. In this article the terms shoal and school were used according to the definition used in Pitcher (1983) and Delcourt and Poncin (2012). A shoal is defined as a group of fish swimming together for social reasons, rather than formed by external factors, while a school is defined as a shoal of fish moving in a synchronized and polarized pattern (Pitcher, 1983; Pitcher and Parrish, 1993; Delcourt and Poncin, 2012). To uphold a dense shoal, fish use a combination of vision, olfaction and the lateral line to locate neighbours while the vision and the lateral line enable schooling (Pitcher, 1979; Partridge and Pitcher, 1980; Faucher et al., 2010). The lateral line enables synchronization in a shoal to form a school, by registration of the water disturbances caused by neighbours to a given individual (Ashraf et al., 2016). Fish derive benefits from shoaling behaviour with increased hydrodynamic efficiency, enhanced foraging success, and defence against predators being main benefits (Hemelrijk et al., 2015).

Individuals will benefit from vortices created by fish swimming straight ahead, by adapting their movements to the vortices and thus decrease resistance and decrease oxygen consumption (Herskin and Steffensen, 1998; Johansen et al., 2010; Hemelrijk et al., 2015).

Fish may increase foraging efficiency with increasing shoal size due to a passive information transfer between the individuals (Pitcher et al., 1982). All individuals in a shoal save energy when shoaling however, leading individuals have a lower amount of energy saving compared to tailing individuals (Killen et al., 2012; Marras et al., 2015). Although shoals often are considered leaderless, some species such as roach (*Rutilus rutilus*) and three-spined stickleback (*Gasterosteus aculeatus*) have shown some degree of leadership (Bumann and Krause, 1993; Krause et al., 2000). Especially the front individuals tend to have a major influence on the direction of the shoal (Krause et al., 2000).

The reaction of the shoal is correlated to how individuals react to certain stimuli. The most common stimuli which would cause a reaction in fish are chemicals, light, pressure, sound, and temperature (Popper and Carlson, 1998). When receiving an intense or unknown stimulus, most species react by fleeing or increase distance from the source of the stimulus (Mork and Gulbrandsen, 1994; Bui et al., 2013). Multiple fish species are susceptible to light and other visual signals due to highly developed light sensory organs (Popper and Carlson, 1998). Schooling behaviour is often most apparent during daylight, due to the requirement of vision to uphold school structure (Didrikas and Hansson, 2008). Most fish species forage actively either during the day or night while only a few species have been observed to feed actively during twilight (Helfman, 1993).

The Atlantic mackerel (*Scomber scombrus*) is an obligate school species known to form huge shoals (Pitcher, 1979) and of high importance for pelagic commercial fisheries. In commercial fisheries mackerel shoals are located by sonar systems (Tennening et al., 2017) that identify reflected pulses of sound energy from hard parts of the mackerel (particularly bones). Sonar systems are also used by fisheries managers for assessment of the stock. Further understanding of mackerel shoaling behaviour might therefore be highly relevant for improving the capture process, supporting reduced carbon dioxide footprint and for fishery managers to estimate number of individuals in the shoal since the “shadow effect” from the acoustical signal depend on the shoal structure.

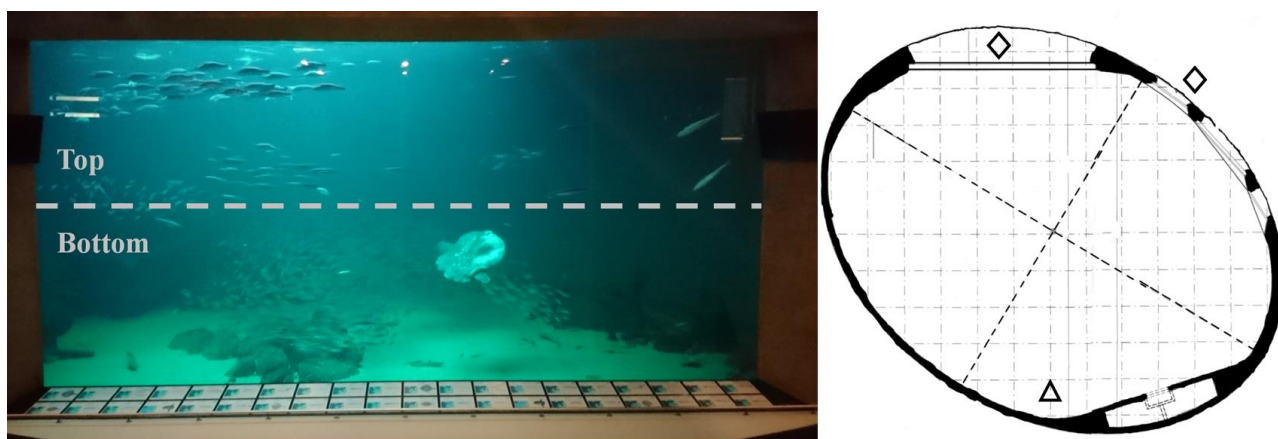
The North Sea Oceanarium, Denmark is a public aquarium exhibiting live aquatic animal and plant specimens of the North Sea. and The Oceanarium holds an Atlantic mackerel shoal in Northern Europe's largest tank (4.5 mio. L. water). This provides a unique opportunity to observe shoal behaviour with some similarity to wild conditions e.g. shoal behaviour in response to feeding. Knowledge of shoal behaviour in an aquarium is of importance to be able to optimise animal welfare as well as educational activities for the visitors.

Ethograms have been used in various studies on species in captivity and in zoos across the animal kingdom such as Arctic charr (*Salvelinus alpinus* (L.)), gorillas (*Gorilla gorilla gorilla*), harbour seals (*Phoca vitulina*), Malayan sun bear (*Helarctos malayanus*), meerkats (*Suricata suricatta*), sitatunga (*Tragelaphus spekii*), and sole (*Solea solea*) among others (Mas-Muñoz et al., 2011; Stevens et al., 2013; Bolgan et al., 2015; Myles and Montrose, 2015; Rog et al., 2015; Račevska and Hill, 2017). Ethograms have been used to investigate reaction to stimuli, the influence of guests, social dynamics, stereotypical behaviour, and general activity patterns (Mas-Muñoz et al., 2011; Stevens et al., 2013; Bolgan et al., 2015; Myles and Montrose, 2015; Rog et al., 2015; Račevska and Hill, 2017). The use of an ethogram in a study on captive sole showed behavioural traits which may be of interest when selecting individuals for breeding programs and when considering welfare and performance of fish in captivity (Mas-Muñoz et al., 2011). In an ethogram, behaviour is categorized into objective mutually exclusive units, which can be used to make a pattern of individual or group behaviour of animals both in the wild and in enclosures or laboratories (Lehner, 1996; Bolgan et al., 2015). The units may be ranked, so the observer know which unit to choose if multiple behaviours occur simultaneously (Cohn and MacPhail, 1996). To our knowledge, this is the first study investigating shoal behaviour and reaction to external stimuli (feeding and non-feeding) using the shoal's position, formation, movement, and density in an ethogram.

## Materials and Methods

### *Fish and fish tank*

Atlantic Mackerel has since 2014 formed a shoal of approximately 1500 adult individuals in the big tank at The North Sea Oceanarium. The ellipse shaped tank (22 x 33 meter) is eight meter deep and surrounded by 4 acrylic panel windows (Figure 1). The light of the tank was partly controlled by a skylight curtain and partly by artificial lighting. The water temperature in the tank was constantly kept around 17-18-degree °C and supplied by input from the Skagerrak Sea next to the aquarium in combination with recirculation. In the tank several objects were positioned as hiding places for the smaller fish species eg. shipwreck and stones (Figure 1).



**Figure 1:** Left: View into the tank from the front window which is 12 meter wide. The grey line illustrates the border between top and bottom. Right: The tank from above. The diamond shows the position of the two cameras, the triangle shows the point where additional feed was thrown

The tank was inhabited with additional 500-1500 fish eg. horse mackerel (*Trachurus trachurus*), garfish (*Belone belone*), plaice (*Pleuronectes platessa*), the greater weever (*Trachinus draco*), six shark species, and two sunfish (*Mola mola*) among others (The North Sea Oceanarium, 2019). The animals were fed daily at 13:00 - 13:30 with mussels, shrimps, herring etc. During feeding a diver was present in the tank. Additional to the main feeding event, commercial size 2 mm fish pellets was thrown into the water from the surface various times a day.

### *Observation protocol*

Two GoPro cameras were positioned in front of the windows to enable view of the shoal from different angles. One Go pro 5 camera (camera 1) was placed on a tripod in front of a window , at 1.2 meter height, 1.2 meter distance and one meter right from the corner, with a measured 90-degree horizontal angle (Figure 1). A GoPro Hero session (camera 2) was placed in the front of the biggest window just left from the middle with a small angle to enable vision of the top water column. The timetable of the protocol is shown on Table 1.

The study was divided into two trials: 1) A non-feeding trial consisting of six observation days where the school was observed between 10:00 and 14:00; 2) A feeding trial consisting of four observation days where the shoal was observed between 11:15 and 15:15 while being fed with 0.2 kg fish pellets at 12:00, 14:00, and 15:00 additional to the regular feeding schedule. The change in time was for practical reason to enable two additional feedings after the main feeding event. The pellets were thrown into the water surface continuously one handful at a time lasting approximately 40 seconds. Water parameters were also monitored such as oxygen level, pH level, light intensity, and temperature (Table 1).

Table 1: Timetable of the setup including the time differences in the trials and used equipment.

Actions	Non-feeding	Feeding	Equipment
<i>Oxygen measurement 1-8 meter depth</i>	09:30	10:45	OxyGuard Handy Polaris 2
<i>1st. pH measurement</i>	09:40	10:55	PH-202 Bench Meter
<i>Camera start</i>	09:55	11:10	
<i>Start</i>	10:00	11:15	ONSET MX Pendant Temp/Light logger
<i>1st. Additional feeding</i>		12:00	
<i>1st. Change of battery</i>	11:30	12:45	
<i>2nd. Change of battery</i>	12:55	14:15	
<i>Feeding</i>	13:00	13:00	
<i>2nd. Additional feeding</i>		14:00	
<i>3th. Additional feeding</i>		15:00	
<i>Stop</i>	14:00	15:15	

#### *Development of ethogram*

An ethogram was designed from data derived from video observations using serial recording method (Cohn and MacPhail, 1996). During the data processing one observer was available to categorize the behaviour and therefore an instantaneous sampling method was chosen (Altmann, 1974). Since this is the first known ethogram used on shoals as one unit, deeper investigations such as behavioural instability have been left out in order to focus on which behavioural category would describe the shoal behaviour most precisely. Thus, during the observation period of four hours one picture was taken from the video data once every minute and the behaviour of the shoal was noted. One observer reviewed the data to ensure the behavioural units were categorized homogeneously. Each picture was observed carefully once and the behavioural units were noted at times the video would run for a few seconds to categorize the movement. During a pilot study four behavioural categories were chosen to represent the shoal behaviour. The first behavioural category monitored was the position of the shoal. The tank was divided in a top and bottom area (Figure 1) to analyse depth preference. The positions were found by locating the majority of the shoal in relation to a horizontal line observed in a 90-degree angle of camera 1. When the shoal was spread equally, the area was noted as top-bottom.

The second behavioural category was formation, which was the morphology of the shoal at a given time. The definitions of all formations used in this study are described in Table 2 and shown in **Error! Reference source not found.** This category included nine behavioural units some inspired by Nøttestad and Axelsen (1999) and Axelsen (2001). Additionally, we defined new categories based on our preliminary observations. Formation was chosen as a category to investigate whether the morphology of the shoal was depended on specific behaviour and reactions. The third behavioural category was movement defined independently of formation and position. Movement was chosen to represent any avoidance and stress behaviour in the shoal. The fourth category was numeric and depending on the shoal's density refereeing to the average body length between a fish and the corresponding fish in the front. The density was chosen to investigate whether density of the shoal depended on certain behaviour and reactions.

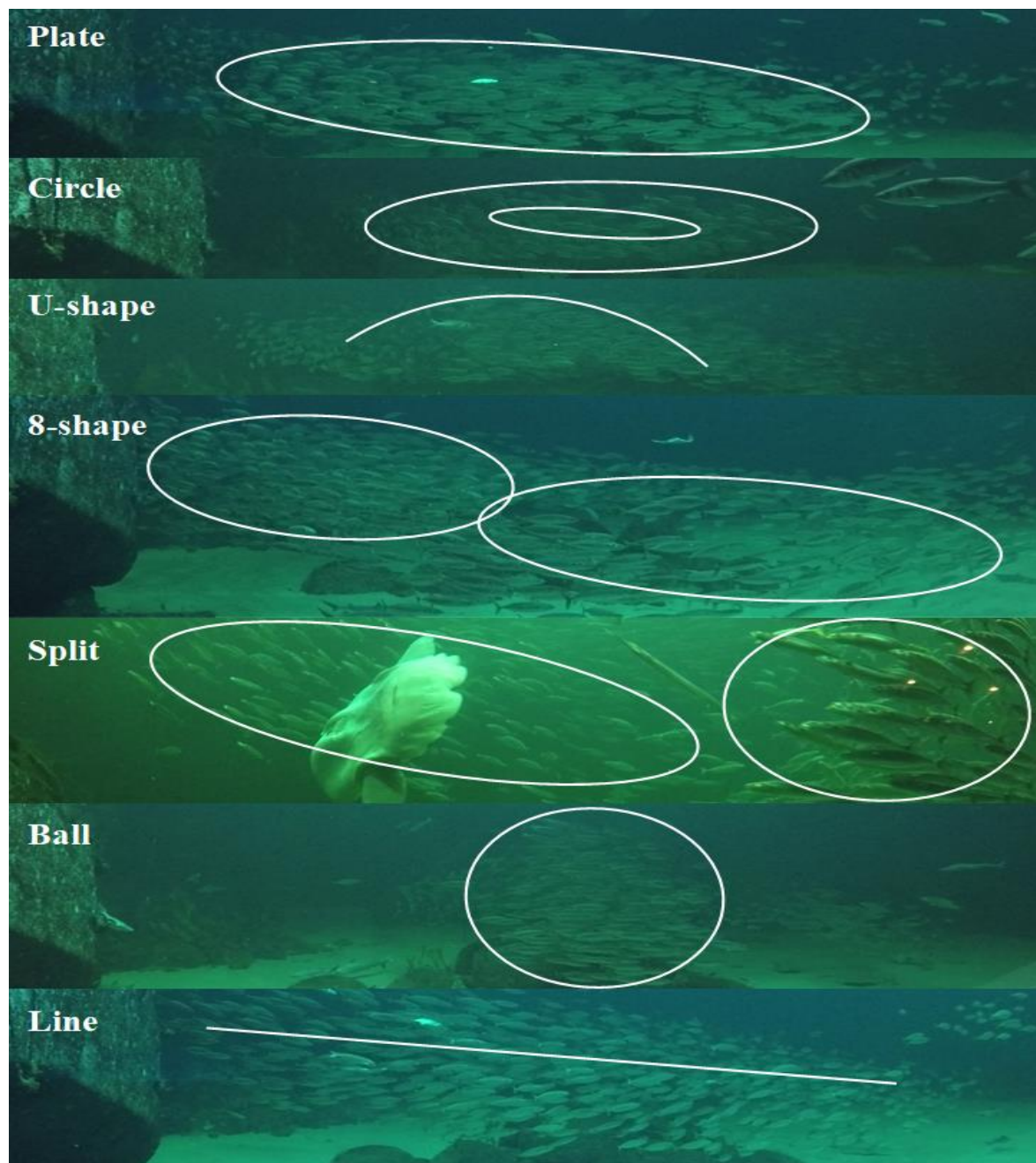
Table 2: Each categorial behaviours used in the investigation, with description and abbreviations in the ethogram.

Category	Behavioural Unit	Description	Abbreviation
<i>Position</i>	<sup>2</sup> Top	The majority of the shoal is positioned in the top half of the tank	T
	<sup>2</sup> Top-bottom	The shoal is evenly distributed between the top and bottom area	TB
	<sup>2</sup> Bottom	The majority of the shoal is positioned in the bottom half of the tank	B
<i>Movement</i>	<sup>2</sup> Forward	Normal movement in the tank	F
	<sup>2</sup> Speeding up	No change in direction, but increased speed	Sp
	<sup>2</sup> Slowing down	No change in direction, but decreased speed	Sl
	<sup>2</sup> Up	Rapid upwards movement	Up
	<sup>2</sup> Dive	Rapid downward movement	D
	<sup>2</sup> Feeding	Food is introduced to the tank and the shoal becomes vague in the pursuit of food	Fe
<i>Formation</i>	<sup>1</sup> Split	The school split into two separate groups to avoid an approaching organism	S
	<sup>1</sup> Vacuole	The shoal is swimming in a ball shape but with a clear hole in the middle because of an obstacle	V
	<sup>1</sup> Hourglass	The shoal is positioned in to levels connected by a narrow hourglass shape of fish either moving up or down	Ho
	<sup>2</sup> Circle	The school is swimming in a circle with a hole in the middle	C
	<sup>2</sup> Plate	Shoal is moving like a circle but with no whole in the middle	P
	<sup>2</sup> 8-shape	Shoal swimming in two circles connected like the number 8	8
	<sup>1</sup> Ball	Fish are concentrated in a ball like structure	B
	<sup>2</sup> Line	The shoal has a front and an end, while the individuals swim head to tail continuedly forward	L
	<sup>2</sup> U-shape	Circle formation breaks do to an organism, where the shoal opens to a u-shape	U
	<i>Density</i>	<sup>2</sup> 0.5 BL	The average length between a front individual and the following member of the shoal is 50 % of the average bodylength (BL)of the fish
<sup>2</sup> 1.0 BL		The average length between a front individual and the following member of the shoal is 100 % of the average bodylength (BL)of the fish	1
<sup>2</sup> 1.5 BL		The average length between a front individual and the following member of the shoal is 150 % of the average bodylength (BL)of the fish	1.5
<sup>2</sup> 2.0 BL		The average length between a front individual and the following member of the shoal is 200 % of the average bodylength (BL)of the fish	2

<sup>1</sup> formations observed in Nøttestad and Axelsen (1999) and Axelsen (2001)

<sup>2</sup> behavioural units observed in pilot studies.





**Figure 2:** Formations displayed by the school. Vacuole is not shown since it was indistinctive from ball in still pictures. The white figures amplifies the differences between the formations.

The ranking of values in the behavioural categories was based on the behavioural unit chosen in the ethogram if there was any doubt which unit should be chosen. One example is the plate or the circle formation which at times were indistinctive, but because the plate is ranked higher (8) than the circle (7), the plate formation was chosen (Table 3). When choosing movement, the shoal may swim upwards in the water column while feeding actively, then feeding was chosen because it was ranked higher (5) than up (3).

*Analysis*

To test whether the designed ethogram had enough units to describe the behaviour for shoal observation fully, a rarefaction curve was drawn with inspiration from Bolgan et al. (2015). When the curves reached a plateau, the ethogram was representative for the shoal’s behaviour. The percentage mean proportion (with standard errors) of observed behavioural units for each observation day were estimated for both trials. In order to test whether the behaviour in the feeding trial and the non-feeding trial were significantly different, a chi-square test of homogeneity (Mann, 2013) was conducted on the four behavioural categories.

Table 3: Ranking of the behavioural units in the categories: formation, area, and movements. The units in the formation category are bold for clarification.

Behavioural category	Position		Formation											Movement							
<i>Behavioural unit</i>	T	TB	B	NM	<b>P</b>	<b>C</b>	<b>U</b>	<b>8</b>	<b>Ho</b>	<b>S</b>	<b>B</b>	<b>V</b>	<b>L</b>	<b>NM</b>	Fe	F	Up	D	Sp	Sl	NM
<i>Numeric value</i>	2	1	0	-1	<b>8</b>	<b>7</b>	<b>6</b>	<b>5</b>	<b>4</b>	<b>3</b>	<b>2</b>	<b>1</b>	<b>0</b>	<b>-1</b>	5	4	3	2	1	0	-1

**Results**

*Rarefaction*

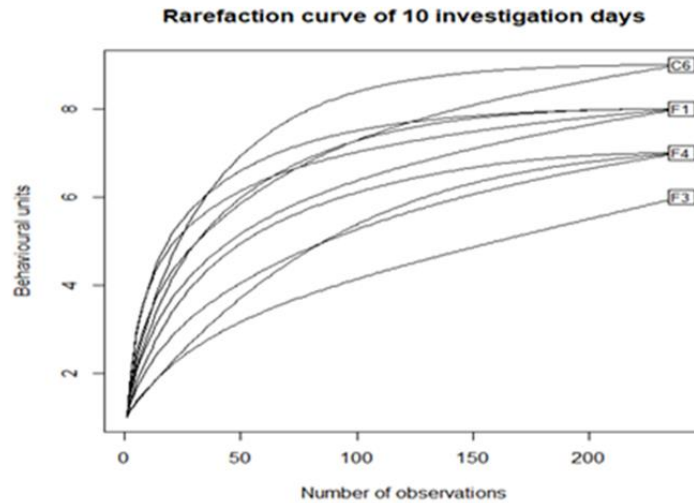
Using the formation units most of the investigation days reached a plateau during the 240 observation point (Figure 3) indicating that the nine chosen values was sufficient for observing the shoal. The oxygen level, pH level, light intensity, and temperature in the tank showed no variation between the trials (data not shown).

*Position*

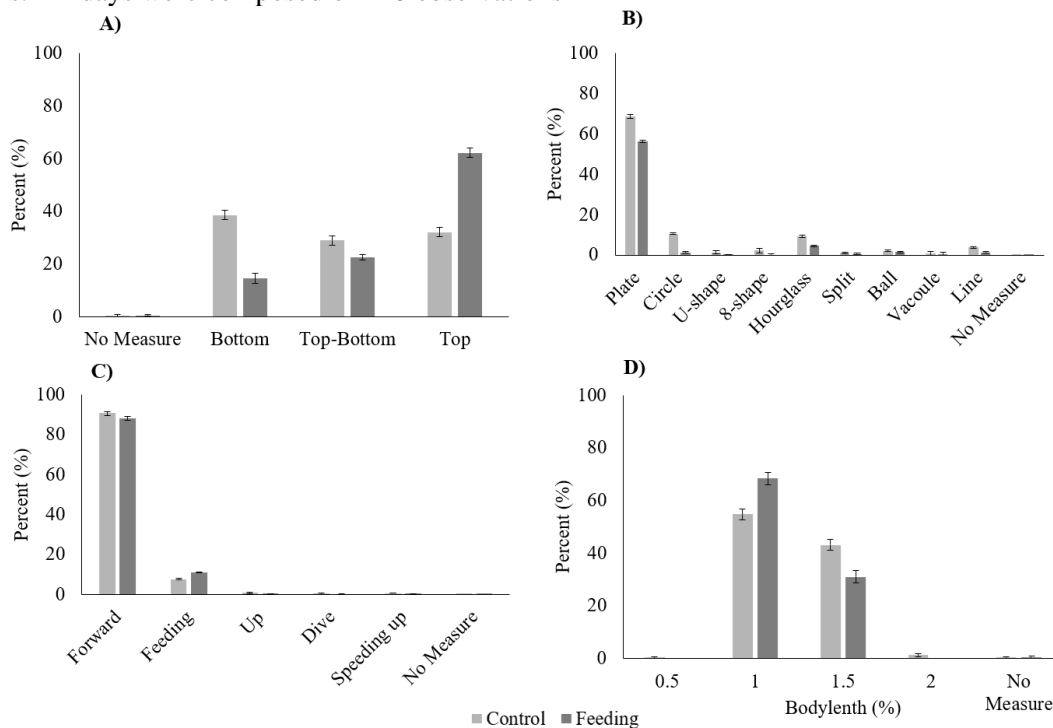
The mackerel shoal was observed in the bottom area  $39 \pm 2\%$  (average  $\pm$  standard error) of the time in the non-feeding trial (Figure 4) and  $15 \pm 2\%$  in the feeding trial. The shoal was more evenly distributed in the top-bottom area in the non-feeding trials ( $29 \pm 2\%$ ), than the feeding trials ( $23 \pm 1\%$ ). During the feeding trial the shoal was in the top area with  $62 \pm 2\%$  of the time and  $32 \pm 2\%$  in the non-feeding trial.

*Formations*

The observation rate of the different formations is shown in Figure 4. The plate formation was observed in  $69 \pm 2\%$  of observations in the non-feeding trial and  $57 \pm 1\%$  in the non-feeding trial (Figure 4). The circle formation was  $11 \pm 1\%$  and  $1 \pm 1\%$  for the non-feeding and feeding trials, respectively. The 8-shape was not observed in the feeding trial, but  $2 \pm 1\%$  in the non-feeding trial. The line formation had an occurrence of  $4 \pm 1\%$  in the non-feeding trial whereas the feeding trial had  $1 \pm 1\%$  of the observations. The hourglass was observed in  $9 \pm 1\%$  of the non-feeding trial and in  $5 \pm 1\%$  in the feeding trial. There were no occurrences of the U-shape, split, and vacuole in the feeding trial, while the ball formation was observed in  $2 \pm 1\%$  of the non-feeding trial and  $1 \pm 1\%$  in the feeding trial. The no measure occurred in both trials with a percentage below one percent.



**Figure 3:** Rarefaction curve of the 10 investigation days. The number of registered behavioural values in the formation category across the investigation days is shown on the y-axis. Number of observations is shown on the x-axis. All days were composed of 240 observations



**Figure 4:** Diagrams illustrating the average percent of observed behavioural categories between the trials. The y-axis shows the percent of occurrence, while the behavioural units are shown on the x-axis. The error bar was based on the standard error of the calculated mean values. A is the position, B is the formation, C is the movement, D is the density



### Movements

The average distribution of the movements across the trials is shown in Figure 4. The feeding trial had  $11 \pm 1$  % feeding movements, and the non-feeding trial  $8 \pm 1$  %. The dive movement was observed in the non-feeding trial with less than one percent, the speeding up was  $1 \pm 0$  % in the non-feeding trial and 0 % in the feeding trial, the up movement  $1 \pm 0$  % in the non-feeding trial and 0 % in the feeding trial, and the no measure movements 0 % in the non-feeding trial and  $1 \pm 0$  % in the feeding trial.

### Density

The average distribution of the density across the trials is shown in Figure 4. The 0.5 BL observation was below one percent in both trials. The 2 BL density was only observed for the non-feeding ( $1 \pm 1$  %). The 1 BL was the most observed density in both trials with  $55 \pm 2$  % of observations in the non-feeding trial and  $68 \pm 2$  % in the feeding trial (Figure 4). The occurrence of the 1.5 BL in the non-feeding trial was with  $43 \pm 2$  % of observations. The occurrence of 1.5 BL in the feeding trial was  $31 \pm 2$  %. The no measure was below one percent in both trials.

### Chi square test

The result of the chi-square test, the degrees of freedom, the rejection region, and the statistic values is shown in Table 4. The movement was concluded to be homogenic in both trials, and therefore not significantly different. In the position, formation and density category, the null hypothesis were rejected so the categories were not homogenic and therefore significantly different in the two trials.

**Table 4:** results of the chi-square test of homogeneity.

Behavioural category	Degrees of freedom	Significance level	Rejection region*	Statistic value $\chi^2$	Null hypothesis
Position	3	0.5 %	12.8	243	rejected
Formation	9	0.5 %	23.6	115	rejected
Movement	5	0.5 %	16.8	14.2	Fail to reject
Density	4	0.5 %	14.9	56.8	rejected

\* the value was taken from Mann 2013

## Discussion

### Capability of the designed ethogram

This investigation on the mackerel shoal is unique compared to other studies, because multiple categories were noted, where the movement category is most similar to the ethogram used in Bolgan et al. (2015). The ethogram produced for this investigation holds a possibility to determine which category is optimal when investigating shoal reactions and general behaviour. The formation category holds the greatest number of units, but the shoal mainly displayed one formation compared to the rest, and the oxygen level, pH level, light intensity, or temperature in this investigation appeared to have no influence on the chosen formation, since the parameters showed no variance between the trials. The movement category included five units but showed the same pattern as the formation. One unit was dominant throughout the observations and none of the units appeared to have an apparent dependency to the monitored parameters. The movement category does have the advantage of monitoring startle responses, and thus may be more useful when studying behaviour. The density category included the smallest number of units, where two was dominant throughout the observations. A low BL appeared to be correlated to foraging behaviour, showed by the increased occurrence of the 1 BL in the feeding trial and the rejected null hypothesis chi-square test for the density, making the observed densities significantly different between the trials. This could be explained by a clustering in the shoal caused by the individuals urge to forage, but a change in density did not follow a clear pattern in this investigation. A high density is generally associated in the wild as a response to threats (Hawkins et al., 2014).

The movement category was the behavioural category used in this investigation with the lowest risk of human error, because of the clear distinction between the units, which made them most likely to be categorised correctly. In this study the movement was shown to be homogenous in both trials and thus not significantly different. The feeding behaviour was a response to food, the forward was the standard motion movement, while the other movement units were initiated rapidly. Further investigation could determine whether the movement units are reactions to external stimuli, e.g. if the up behaviour is a response to disturbance in the surface or the speeding up is a response to the appearance of a threat. Further investigations are needed, but if speeding up and diving could always be categorised as a startle response, the proportion of those movement in a given shoal could indicate the stress level in the shoal. If the shoal speeds up and/or dive and then resume the forward motion repeatedly, it could indicate the shoal being on high alert for threats and in a general stressful condition.

In other investigations, rapid diving has also been defined as a startling response (Popper et al., 1998; Bui et al., 2013; Hawkins et al., 2014). When studying startle responses in fish the concentration of plasma cortisol has been used to determine the stress level during the responses (Richards et al., 2007). It could be interesting to compare the plasma cortisol concentration with chosen movement units in a smaller scale study, in order to verify whether movements could be a less expensive method to categorise stress level in fish living in confinement.

In Bolgan et al. (2015) an ethogram was built up as an identification key in addition to the more traditional method describing behaviour of the arctic charr, where only one behavioural category based on three types with several units in each. These were locomotion, stationary, and social behaviour, but only one unit was chosen each time. Using the key, the observed behaviour was categorized by the process of elimination ensuring the exclusion of the behavioural units from each other (Bolgan et al., 2015). The ethogram used on the arctic charr was more simply constructed, since only one behavioural category was noted. Focusing on one category and elimination options when determining the unit using an identification key was shown to minimize errors in the data compared to the traditional observation method (Bolgan et al., 2015). Another ethogram made for the South European Toothcarp (*Aphanius fasciatus*) was produced in a similar way as to the ethogram in this investigation on the mackerel shoal, but was limited to reproductive behaviour in individuals (Cavrao et al., 2013).

#### *Mackerel shoal behaviour*

In the feeding trial the behavioural categories except for the movement appeared to be less diverse compared to the non-feeding trial. Forward and feeding movements occurred more abundantly in the feeding trial compared to the non-feeding trial. In the formation category the plate also had the highest abundance in the feeding trial compared to the non-feeding with 85 % of occurrence of observations. This indicates that the shoal showed a stable behaviour when fed regularly during the day. When observed in the wild, herring shoals displayed a variety of amorphous formations and would move in various directions (Nøttestad and Axelsen, 1999; Axelsen et al., 2001). This may be due to a foraging behaviour, where the shoal investigates the waters for food, which was not possible for a shoal in an aquarium.

This mackerel shoal observation was consistent with the findings of Axelsen et al. (2001) where large groups of herring were observed to prefer an epileptically shape next to an amorphous shape. The proportion of plate observations was high, but the measured proportion may not be as high as the actual proportion due to the limited visibility from the cameras. An edged opening might appear on the opposite side of the shoal as the camera, or the dense shoal may disguise an opening in the middle. The plate was the highest ranked formation unit and would be chosen over the other formations, if a formation could not be described with certainty. This could lead to a higher notion of the plate formation compared to the real proportion.

The feeding stimuli introduced to the shoal lasted under a minute with each inducement. This left the majority of the observation time in the feeding trial to be similar to the non-feeding trial. This does however show, there is no clear long term reaction from the shoal by additional feeding, though the shoal was observed at a higher rate in the top area in the feeding trial.

In this investigation the forward movement was categorised as the normal speed of the shoal. The precise speed was not measured, so a speeding up or slowing down had to be abrupt and clear in the video data before it could be categorised and was categorized while the video was running. Therefore, small changes in speed was not included and could explain the lack of the shoal slowing down compared to the normal speed. If more time had been available and a continuous focal sampling had been chosen, the movement units might have been more prominent compared to the instantaneous method. The continuous focal sampling have been used in studies on wild geese and captive polar bears with focus on behavioural instability as a novel approach (Bech-Hansen et al., 2019; Linder et al., 2020). Behavioural instability as a stress indicator could be a useful application in future studies on shoal behaviour using the movement category in a more specified ethogram. If a shoal at one observation day generally moved faster than another, this would be categorised as the normal speed. Thus, when speeding up was observed, it was likely to have been a startle response in this investigation, due to the sudden visible change in behaviour. Diving would also only be categorised if the individuals in the shoal changed the angle of the body to dive into lower areas, in a sudden movement. The shoal has been observed to gradually move from the top to the bottom, but the body angle of the individuals would not change compared to forward movement and would then be categorised as forward.

#### *Perspectives of the setup*

With two cameras monitoring the shoal from different angles, it was possible to observe the behaviour, but the visibility was still limited due to the shape of the tank, the light bending in the window panels, and obstacles in the tank and in front of the windows. Although the observed tank was the largest aquarium in Northern Europe and provide an opportunity to investigate a full-size shoal swimming in various formations and patterns, the shoal was still stationary and inhibited by vertical barriers in contrast to wild shoals. This may force the shoal into the circular formations as plate, circle and 8-shape at higher proportions, compared to the wild. A wild shoal may show a higher proportion of a line formation or other amorphous shapes in the open ocean. Another uncertainty was whether the shoal had been accustomed to the tank including human activity e.g. diving and feeding over several years. The shoal approached the surface when food and other objects were thrown into the water, instead of fleeing from a possible avian predator. The shoal also approached the diver, because the diver was associated with food, and not an unknown threat. During the investigation no active predation on the shoal has been observed, even though the shoal acted and avoided larger fish if they approached the shoal. This adaptation may have reduced predator avoidance behaviour, which is one of the main benefits of shoaling. If the avoidance behaviour was reduced in the tank, a wildlife shoal's behaviour and distribution of behaviour may be significantly different. Especially the ball formation may be observed more often, as other shoals tend to show this formation when predated on as seen in Nøttestad and Axelsen (1999) and Axelsen et al. (2001).

#### *Use of the investigations*

Findings suggest that it is possible to observe a number of different behaviour patterns where some are comparable to field study observations from the sea. But some limitations in natural behaviour patterns caused by physical barriers in confinement limits the comparison to wild conditions. This offers the opportunity for the audience in the aquarium to understand shoaling behaviour. Behavioural studies have been used in welfare studies on mammals in zoo and may also have a relevance for confined fish welfare. In relation to exploitation of mackerel in commercial fisheries, the shoal formation and density will likely influence the estimate of the shoal size and number of individuals in the school estimated by an acoustic signal. Integrating this information to algorithms in sonar systems could have a significant impact.

## Acknowledgments

The authors want to thank the North Sea Oceanarium for providing access to their mackerel shoal enabling this study and to Martin Riis and the other staff members at the Oceanarium for help during the study.

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