



Learning and anticipatory behaviour in a “sit-and-wait” predator: The Atlantic halibut

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ABSTRACT

We studied the learning capacities and anticipatory behaviour in a “sit-and-wait” predatory fish, the Atlantic halibut, *Hippoglossus hippoglossus*. In Experiment 1 two groups of halibut received series of light flashes (conditioned stimulus, CS) that started before delivery of food (unconditioned stimulus, US) and persisted until after food delivery, i.e. delay conditioning. Control groups received unpaired CS and US presentations. The anticipatory behaviour of delay conditioned halibut consisted mainly of take-offs towards the surface shortly after onset of the CS. In Experiment 2 six groups of halibut were trained in three trace conditioning procedures: Two groups with 20 s, two groups with 60 s and two groups with 120 s trace interval. Learning was evident in the 20 and 60 s trace groups and in one of the 120 s trace groups. In contrast to delay conditioning the anticipatory behaviour of trace conditioned halibut was characterized by subtle movements near the tank floor with orientation towards the CS. The cautious responses of halibut after trace conditioning differed markedly from what is observed in other fish species and are suggested to reflect a “sit-and-wait” foraging strategy that requires the predator to remain undetected until the prey is within lunging range.

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1. Introduction

The past decade has seen a wealth of studies on fish learning (Brown et al., 2006). However, studies on the mechanisms involved in learning are scanty. For studies of fish ecology it is crucial that we gain more insight into the learning capacity and constraints in different species. One context in which learning plays an important role is foraging (Croy and Hughes, 1991; Warburton, 2006). A type of learning that has a significant impact on the ability to recognize and respond to prey is Pavlovian (classical) conditioning (Lieberman, 2000), i.e. associations between initially neutral cues (conditioned stimulus, CS) and biologically relevant stimuli such as food (unconditioned stimulus, US). For fish, stimuli that announce a greater probability of encountering food could involve visual features of the prey itself or cues that occur together with prey, e.g. sounds or odours. Such cues may increase attention and preparedness to attack, and thus increase capture success. The anticipatory behaviour, i.e. the form and timing of the response, may be expected to reflect not only the informational value of the cue (e.g. type of prey, temporal and spatial relationship between

cue and prey encounter), but also the foraging strategy of the predator.

Cues and prey encounter often overlap in time, i.e. the cue persists until the prey is detected and caught (delay conditioning). In other occasions the cue may disappear before the prey is detected (trace conditioning), for instance prey-induced movements of vegetation or sediment. Most of the experimental works on Pavlovian learning in fish have used delay conditioning, and it has long been known that fish rapidly learn to associate different cues with rewards (Bull, 1928). Little, however, is known about the abilities of trace conditioning in fishes. In a recent study we found that groups of Atlantic cod, *Gadus morhua*, could be conditioned to associate a light signal in the feeding area with a food reward at trace intervals as long as 120 s (Nilsson et al., 2008a), demonstrating that some fish have excellent capacities for trace conditioning.

Once an association between a CS and a rewarding US has been formed, the CS alone elicits a response. The response often reflects stimulus substitution, where subjects direct their behaviour to the CS, e.g. by approaching it or even trying to catch or ingest it (Brown and Jenkins, 1968; Purdy et al., 1999), a behaviour referred to as sign-tracking (Hearst and Jenkins, 1974). For instance, archer fish, *Toxotes chatareus*, respond to a CS light above the surface by squirt-water at it when it is paired with fruit flies delivered on the surface (Waxman and McCleave, 1978). Cod always approached

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the CS light, which was located on the opposite side of the feeding site in a 3 m tank, before they gathered in the feeding area prior to food arrival (Nilsson et al., 2008b). A hunting cod generally orients to and approaches prey upon detection (Brawn, 1969; Steingrund and Fernö, 1997). Sign-tracking may thus lead to efficient responses to cue signals for cod and other species with similar foraging strategies. Cue-induced anticipatory responses in fishes have been studied little, and not much is known about learning capacities and anticipatory responses in fish with other foraging strategies, such as “sit-and-wait”.

In “sit-and-wait” ambush strategies, an immediate approach response (sign-tracking) to food-announcing cues may not be adaptive, for at least two reasons. First, rapid approaches could frighten away prey that is out of lunge range. Secondly, the place where a cue is detected may not be the same as where the prey is caught. As a successful “sit-and-wait” ambush requires attack at a suitable distance and angle, cues should be expected to induce increased attention and adjustments of body posture and position rather than release immediate attack-like responses. The spatial and temporal relationship between the cue and the associated prey should then determine the form and timing of the response, i.e. which behavioural components that are involved in the response and at which stimuli these are directed, as well as when to respond. In fact, the “sit-and-wait” predator domestic cat, *Felis silvestris catus*, becomes hypoactive while the rat, *Rattus norvegicus*, that applies a “search behaviour”, becomes hyperactive in the same appetitive conditioning procedure with the different anticipatory responses reflecting their foraging strategies (van den Bos et al., 2003).

Flatfish are “sit-and-wait” ambush predators (Gibson, 2005), famous for their unusual morphology and excellent camouflage, but little is known about their learning abilities. We studied the capacity for associative learning and anticipatory behaviour in the Atlantic halibut, *Hippoglossus hippoglossus*. Halibut is the largest flatfish species and may reach a weight of >300 kg and ages of over 50 years (Haug, 1990). Small individuals live quite localized on sandy bottoms at moderate depths (20–60 m) and feed mainly on benthic crustaceans, while fish become more important prey as the halibut grow (Haug, 1990). Prey detection relies both on vision and olfaction (de Groot, 1969; Yacoob and Browman, 2007). When the distance to the prey is short enough prey are captured in a rapid lunge with simultaneous opening of the mouth and protrusion of the jaws, with the prey being sucked into the mouth (Gibson, 2005).

With a feeding strategy that relies on camouflage, ambush and surprise, we hypothesised that halibut should not react to reward-associated cues by immediate approach responses or sign-tracking, but rather prepare an attack by more subtle responses. In Experiment 1 we used delay conditioning to study learning and anticipatory response with overlapping CS–US presentations and a relatively short CS–US interval. In Experiment 2 we used trace conditioning with three different trace intervals to examine whether halibut have the capacity of trace conditioning, and if so, whether the anticipatory behaviour is expressed differently than during delay conditioning.

2. Experiment 1—delay conditioning

2.1. Methods

2.1.1. Experimental setup

Four circular 1 m diameter tanks of black plastic were used for the experiment. To facilitate a smooth water exchange and removal of uneaten food and faeces by the exchanged water, the tank floor was kept plain without substrates or other structural features. The tanks were filled with 60 cm seawater (≈ 450 L) at 12 °C and 90% O₂

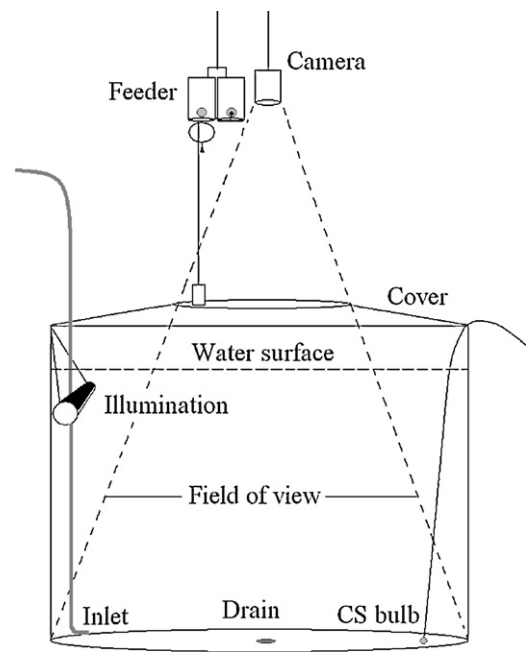


Fig. 1. The experimental setup (side view).

saturation, and continuously illuminated by underwater fluorescence tubes (33 lx). The water was exchanged at a rate of 10 L min⁻¹. A video camera hung above each tank. A cover with a 60 cm diameter hole was placed over each tank to prevent escape of fish from the tanks and to avoid direct light from the fluorescence tubes on the recordings. The field of view of the camera thus did not cover the upper sides of the tanks (Fig. 1). A 3 W light bulb was placed on the floor of the tank about 10 cm from the wall opposite to the illumination. Food was delivered at the surface from a feeder. Uneaten food was removed through the drain within a few minutes.

2.1.2. Fish and food

Four groups of 20 one-year-old halibut, hatched in captivity from eggs from wild-caught parents, were measured for length (15 ± 1.0 cm, mean \pm S.D.) and allowed to recover in the experimental tanks for 9 days in advance of the experiment. When lying on the black tank floor halibut attained a skin pattern of white spots and a darker brown colour than when swimming off the floor, reflecting an attempt to camouflage. Halibut have low motivation to eat common formulated food (Kristiansen et al., 2004), and a pilot conditioning experiment revealed that formulated food often fails to induce immediate feeding response and is thus not appropriate as reward (own unpublished observations). Shrimp, *Pandalus borealis*, induces strong feeding behaviour in juvenile halibut (Yacoob and Browman, 2007), and boiled and chopped-up shrimp was used as reward in the experiment. On the first introduction of shrimp halibut did not respond immediately at the sight of shrimp, but a strong response similar to that reported by Yacoob and Browman (2007) occurred after around 10 s and first feed intake after around 15 s. This indicates that they mainly responded to the olfactory cue. In order to facilitate familiarity with this food and ensure that halibut also responded to the sight of shrimp, they were fed shrimp instead of formulated food the last 3 days before the start of the experiment. On the last feeding sessions before the start of the experiment the time to first feed intake was reduced to around 7 s. At delivery, the shrimp spread out on the surface, and sank at a rate of about 6 cm s⁻¹ following the slow clockwise flow in the tank.

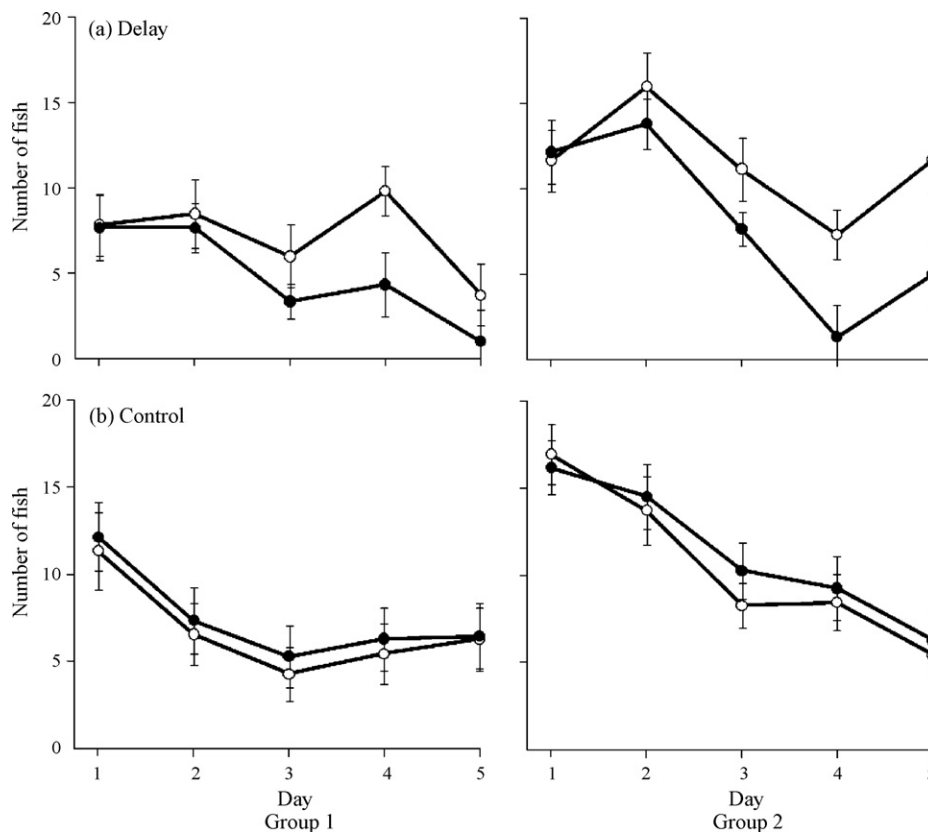


Fig. 2. Mean \pm S.E. number of fish maintaining their positions on the floor of the tank during a 10-s period immediately before the onset of the CS (open circles) and throughout the first 10 s of the CS (the CS–US interval in the delay procedure, filled circles) in (a) the delay procedure and (b) the control procedure. Left and right figures represent replicate groups.

2.1.3. Procedures

For two groups each feeding was announced by a series of light flashes (conditioned stimulus; 1 s on:1 s off) from the light bulb. The CS had a duration of 20 s and started 10 s before delivery of shrimp (unconditioned stimulus), i.e. 10 s overlapping with the US (delay conditioning). In two unpaired control groups shrimp was delivered 1 h after CS presentation. The scheduled procedure was six trials per day with 2-h intertrial intervals. Due to a few deviations from the planned schedule, with for instance the CS bulb not working, the average number of trials per day was 5.6 (range: 4–6). The fish received these schedules for 5 days. On days 6–8, when a response had been acquired in the delay groups, the duration of the CS was increased to 40 s (starting 30 s before food delivery in the delay procedure) in order to provide longer observation times of the anticipatory behaviour. The trials were recorded on DVD for subsequent analysis.

2.1.4. Behavioural analysis

Halibut usually spend most of the time lying motionless on the bottom. Our prediction was that anticipatory behaviour induced by the CS would make the halibut change position more often. For all trials on the first 5 days of the experiment, the position of the snout of each fish was registered on the video image immediately before the CS, and the number of fish that remained in position 10 s later (i.e. immediately before food delivery in the delay groups) was recorded. To estimate the baseline level of motion the same procedure was made for a 10-s interval immediately before the CS (pre-CS). While this analysis did not give any details of how and when the halibut responded, the form and temporal distribution of responses were studied in more detail in all trials on the last day of the experiment (day 8), when the CS duration had been extended.

A time period starting 20 s before and ending 30 s after the onset of the light flashes (i.e. at food delivery in the delay groups) was divided into 5-s intervals, and the number of fish lying motionless at the end of each 5-s interval was recorded. We also registered how many times the following three behavioural patterns were initiated in each 5-s interval: *Take-off*: Lift from the bottom and swim in the water column for more than 10 s; *Bottom-swim*: Moving more than three body lengths along the bottom; *Reposition*: A small move, less than three body lengths, usually straight forward or a turn.

Whether fish approached the CS (sign-tracked) was determined on the basis of analyses of days 6–8. The image of the tank on the screen was divided into four equal 90° sectors, with the CS light bulb in the centre of one sector (CS sector), and the number of fish on or immediately above the floor in the CS sector was recorded 5 s before and 10 s after the onset of the CS. In order to determine whether fish directed their attention towards the CS without approaching it, the number of fish on or immediately above the floor with their heads directed $\pm 45^\circ$ towards the CS light bulb was recorded on the same images.

2.1.5. Statistics

The data resulting from the video analysis were categorical variables that indicated the frequency of different behaviours. As it was difficult to confirm or transform these variables into a normal distribution, nonparametric statistical methods were used. An anticipatory response to the CS should lead to fewer fish maintaining their positions on the tank floor during the CS–US interval as compared to before the CS, i.e. a change from the pre-CS level. Kendall tau rank correlation was used to test if there was a correlation between the magnitude of the change and trial

Table 1
Probability levels given by the aligned ranks test for randomized complete blocks on the effect of period (pre-CS, 1st third, 2nd third and last third of the CS–US interval) on the number of motionless fish, take-offs, bottom-swims and repositions in the delay procedure and the control procedure. The column “Main effect” gives the probability level when the entire dataset (all four periods) was included. The columns “1st third”, “2nd third” and “Last third” give the probability level when each of these periods was compared with pre-CS. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s., not significant. Note that the effects on “Motionless” are due to a decrease in number of motionless fish during the CS–US interval in the delay procedure, while it is due to an increase in the control procedure.

Behaviour	Procedure	Group	Main effect	1st third	2nd third	Last third
Motionless	Delay	1	***	***	***	***
		2	***	***	***	***
	Control	1	***	**	***	***
		2	***	*	***	***
Take-off	Delay	1	*	**	n.s.	n.s.
		2	***	**	n.s.	n.s.
	Control	1	n.s.			
		2	n.s.			
Bottom-swim	Delay	1	**	***	**	**
		2	n.s.			
	Control	1	n.s.			
		2	n.s.			
Reposition	Delay	1	n.s.			
		2	n.s.			
	Control	1	n.s.			
		2	n.s.			

number, indicating learning. Wilcoxon signed rank test was used to compare the number of fish in the CS sector and the number of fish oriented towards the CS bulb before and during the CS. For the observations on the last day of number of motionless fish, take-offs, bottom-swims and repositions in 5-s intervals, an aligned ranks test for randomized complete blocks (Stokes et al., 2000) was used. In short, each procedure was divided into four periods, pre-CS, first third, second third and last third of the CS–US interval, and the test identified if there were significant effects from these periods for each behavioural pattern. The test was first performed using the entire dataset, and if an effect was found the test was performed on each of the three CS–US periods at a time to detect differences from pre-CS levels. All tests were performed separately for each replicate group. All tests were two-tailed and the level of significance was set at 0.05.

2.2. Results

2.2.1. Response acquisition

There was little response in the delay groups to the CS on the first day (Fig. 2a). On the subsequent days the number of fish remaining motionless on the floor decreased during the CS compared to pre-CS level, and the magnitude of the decrease was correlated with trial number (Group 1: $\tau = -0.37$, $p < 0.01$; Group 2: $\tau = -0.57$, $p < 0.001$). In the control groups there was no significant correlations (Group 1: $\tau = -0.06$, $p > 0.05$; Group 2: $\tau = 0.23$, $p > 0.05$), and the change from pre-CS level to CS was generally small (Fig. 2b).

2.2.2. Form of the response

In the delay groups the effect of period (pre-CS, first third, second third and last third of the CS–US interval) on the number of motionless fish was significant, with the number of motionless fish lower

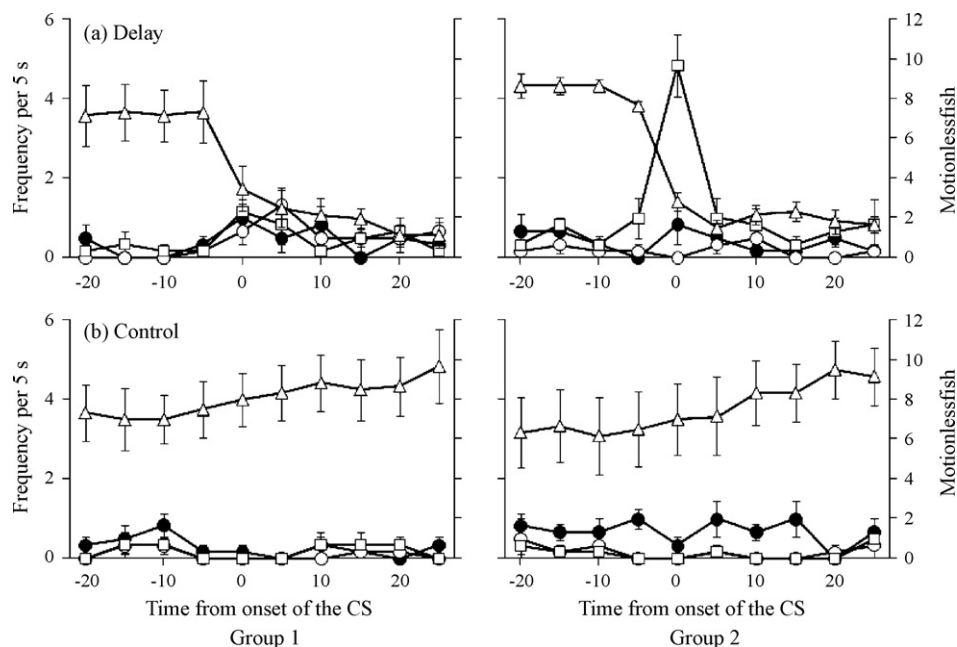


Fig. 3. Behaviour of groups of halibut in relation to time from onset of the CS. Triangles: number of fish lying motionless on the floor at the end of each 5-s interval (x -value is the first second of the interval, e.g. the interval “0” is 0–5 s from onset of the CS); filled circles: repositions; open circles: bottom-swims; squares: take-offs. Mean \pm S.E. values based on the final six conditioning trials day 8. (a) delay procedure and (b) control procedure. Left and right figures represent replicate groups.

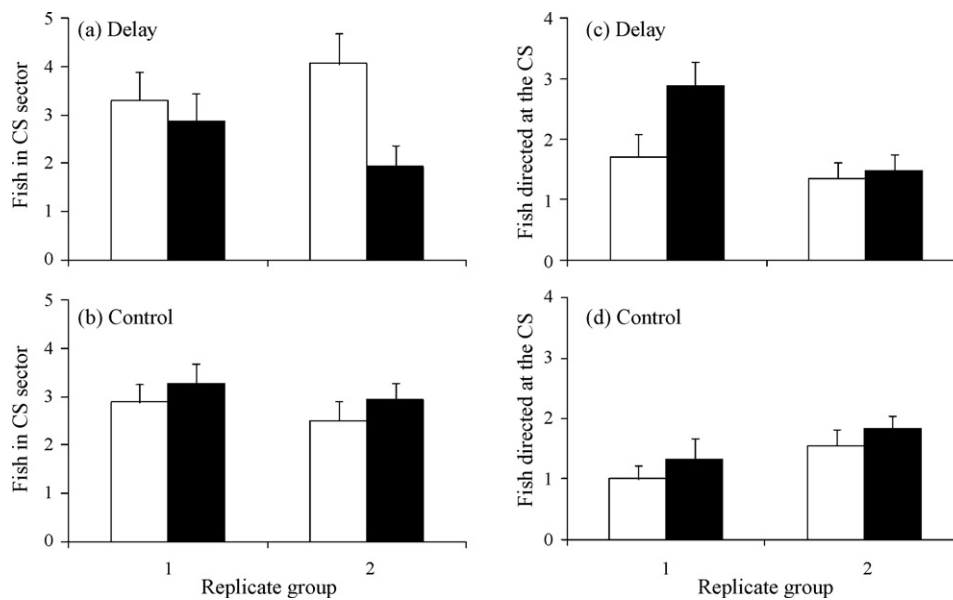


Fig. 4. Sign-directed behaviour of halibut. Open bars: 5 s pre-CS; filled bars: 10 s after the onset of the CS. Number of fish in the CS sector in (a) the delay procedure and (b) the control procedure, and number of fish directed $\pm 45^\circ$ towards the CS bulb in (c) the delay procedure and (d) the control procedure. Mean \pm S.E. based on all (17 for the delay groups, 18 for the control groups) trials on days 6–8.

than pre-CS level throughout the CS in both groups (Table 1 and Fig. 3a). *Take-off* was the main response, and most take-offs occurred during the first seconds after the onset of the CS (Table 1 and Fig. 3a). The take-off response was especially dominant in Group 2, in which most of the fish swam near the surface after take-off. Group 1 also responded with *bottom-swim*, which occurred more often throughout the CS than pre-CS (Table 1 and Fig. 3a). The rate of *repositions* during the CS did not differ from pre-CS level (Table 1 and Fig. 3a).

In contrast to in the delay groups, the number of motionless fish in the control groups increased slightly throughout the CS (Table 1 and Fig. 3b). None of the three behavioural patterns occurred at a higher rate during the CS than pre-CS (Table 1 and Fig. 3b).

2.2.3. Sign-tracking

The delay groups did not sign-track by moving to the CS. The number of fish in the CS sector was low during the CS in both groups and did not differ from pre-CS level in Group 1 ($T_+ = 34.5$, $p > 0.05$), while the number decreased in Group 2 ($T_+ = 113$, $p < 0.01$, Fig. 4a). However, the number of fish directed towards the CS light bulb slightly increased during the CS in Group 1 ($T_+ = 9$, $p < 0.01$, Fig. 4c), while there was no difference in Group 2 ($T_+ = 48$, $p > 0.05$).

In the control groups the number of fish in the CS sector was slightly higher during the CS than pre-CS in Group 2 ($T_+ = 0$, $p < 0.05$), but not significantly so in Group 1 ($T_+ = 0$, $p > 0.05$, Fig. 4b). The average increase in the CS sector (25% of the tank floor) was 0.39 and 0.44 fish for Groups 1 and 2, respectively, and is thus in accordance with the total increase on the tank floor of 1.33 and 1.83 (see Fig. 3b). The number of fish directed towards the CS light bulb during the CS did not change from pre-CS level (Group 1: $T_+ = 2$, $p > 0.05$; Group 2: $T_+ = 10$, $p > 0.05$, Fig. 4d).

3. Experiment 2—trace conditioning

Experiment 1 demonstrated that halibut can rapidly become conditioned when trained with overlapping CS–US presentations. Contrary to our hypothesis that anticipatory behaviour would be characterized by subtle movements, the main response was to lift from the bottom and swim actively in the water column, more similar to an ongoing attack of prey than an attempt to prepare for a future attack. An explanation for this could be that the halibut

were trained with overlapping CS–US presentations and a relatively short CS–US interval, a CS–US relationship resembling situations where the reward is or will soon be within range. Then there would be little time and little reason to avoid being detected by the prey.

In a situation in which a “sit-and-wait” predator gets a cue about the presence of a prey before the prey is available for attack, e.g. out of a halibut’s vision or lunge range, a more cautious behaviour may be crucial to come within range without alarming the prey. Trace conditioning, in which the CS is terminated before the presentation of the US, would resemble such a situation. In Experiment 2 we therefore trained groups of halibut at three different trace interval durations, 20, 60, and 120 s, in order to study if (a) the anticipatory behaviour of halibut is expressed differently during trace conditioning than delay conditioning and (b) halibut has the capacity to be trace conditioned at long trace intervals and if the anticipatory behaviour is affected by the duration of the interval.

3.1. Methods

The CS duration was 10 s, and the offset of the CS was separated from the US with a trace interval of 20, 60, or 120 s with two replicate groups of 20 halibut for each trace duration. The CS–US interval (the CS and the trace interval) was thus 30 s or more, giving sufficient time to analyse anticipatory responses. The length of the fish was 18.7 ± 1.1 cm (mean \pm S.D.) in the 20 and 60 s trace procedure and 21.3 ± 1.4 cm in the 120 s trace procedure, which were carried out 3 weeks later than the 20 and 60 s procedures. As learning was assumed to be slower with trace conditioning, which is more demanding than delay conditioning (Lieberman, 2000), the fish were trained for 12 days instead of 5 in Experiment 1, with on average 5.8 trials per day. The setup, procedure and analyses were otherwise the same as in Experiment 1.

3.2. Results

3.2.1. Response acquisition

20 s trace groups: With the exception of the first day the number of fish remaining motionless on the floor decreased during the CS–US interval compared to the pre-CS level, and on the last day very few fish remained motionless throughout the CS–US interval

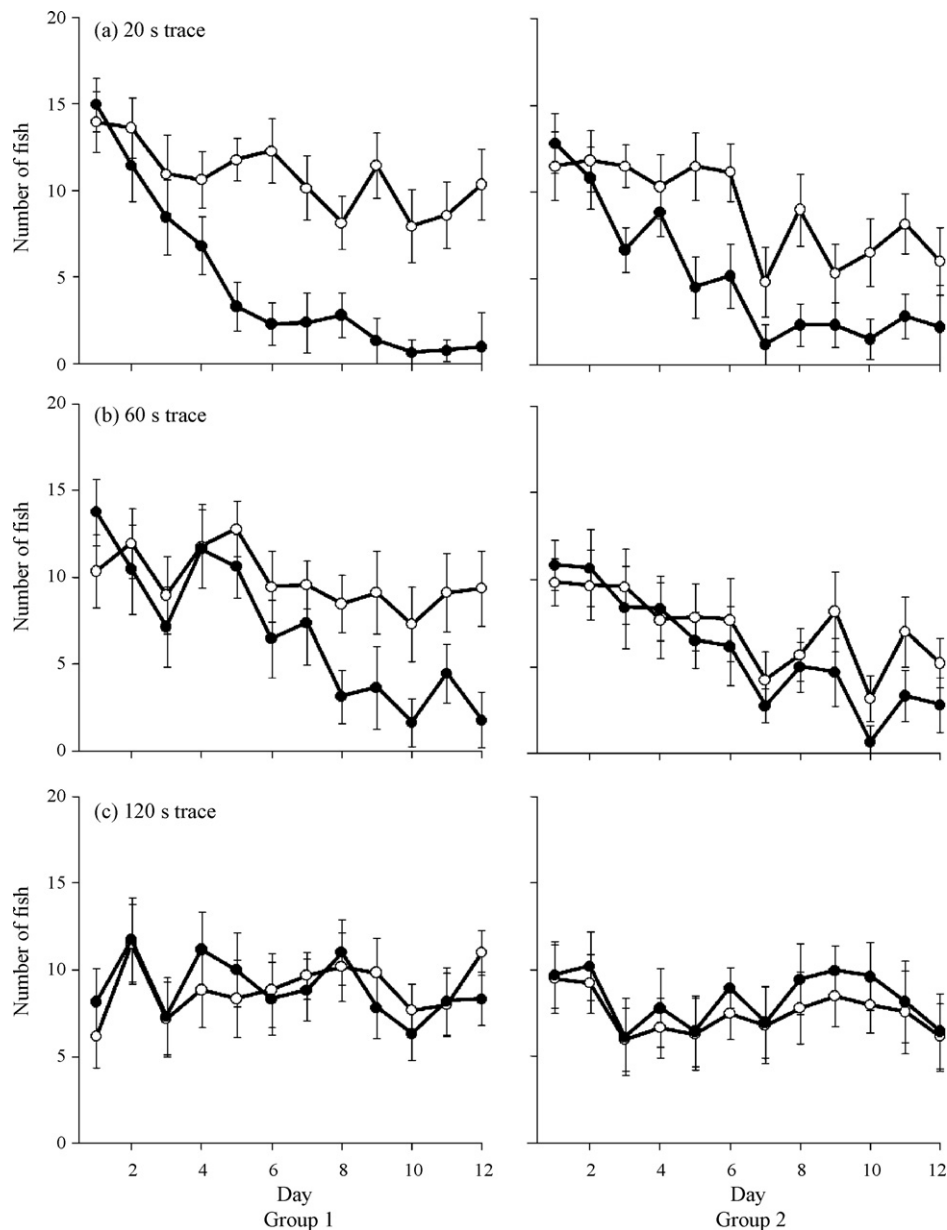


Fig. 5. Mean \pm S.E. number of fish maintaining their positions on the floor of the tank throughout the CS–US interval (filled circles) and through an equal-length period immediately before the onset of the CS (open circles) in (a) the 20 s trace procedure, (b) the 60 s trace procedure, and (c) the 120 s trace procedure. Left and right figures represent replicate groups.

(Fig. 5a). The magnitude of the change from pre-CS to the CS–US interval was correlated with trial number (Group 1: $\tau = -0.44$, $p < 0.001$; Group 2: $\tau = -0.26$, $p < 0.01$).

60 s trace groups: The difference between the CS–US interval and pre-CS in number of fish remaining motionless was less pronounced and the response acquisition slower (Fig. 5b) than in the 20 s trace groups. Still, on the last day few fish remained motionless throughout the CS–US interval, and the magnitude of the change from pre-CS to the CS–US interval was correlated with trial number (Group 1: $\tau = -0.46$, $p < 0.001$; Group 2: $\tau = -0.42$, $p < 0.001$).

120 s trace groups: The difference between the CS–US interval and pre-CS in number of fish remaining motionless was small in both groups throughout the experiment (Fig. 5c). In Group 1 the number of motionless fish was higher during the CS–US interval than pre-CS on the first day and lower on the last day (Fig. 5c), with a correlation between the magnitude of the change and trial number ($\tau = -0.35$, $p < 0.001$). In Group 2 the number of motion-

less fish was slightly higher during the CS–US interval throughout the experiment (Fig. 5c), and the magnitude of the change was not correlated with trial number ($\tau = 0.03$, $p > 0.05$).

3.2.2. Form of the response

20 s trace groups: The number of motionless fish fell after the onset of the CS and remained lower throughout the CS–US interval (Table 2 and Fig. 6a). The main response to the CS was *repositions*. The rate of repositions showed a peak in the first seconds following the onset of the CS, though it was higher than pre-CS level also later in the CS–US interval (Table 2 and Fig. 6a). *Bottom-swims* were less frequent than reposition, but more frequent than pre-CS level throughout the CS–US interval in Group 1, while the rate during the CS–US interval did not differ from pre-CS level in Group 2 (Table 2 and Fig. 6a). *Take-offs* were relatively rare during the CS–US interval in both groups (Fig. 6a), but more frequent than pre-CS level in Group 1. In Group 2 there was no difference (Table 2).

Table 2

Probability levels given by the aligned ranks test for randomized complete blocks on the effect of period (pre-CS, 1st third, 2nd third and last third of the CS–US interval) on the number of motionless fish, take-offs, bottom-swims and repositions in the 20 s trace, 60 s trace and 120 s trace procedures. The column “Main effect” gives the probability level when the entire dataset (all four periods) was included. The columns “1st third”, “2nd third” and “Last third” give the probability level when each of these periods was compared with pre-CS. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s., not significant.

Behaviour	Procedure (s)	Group	Main effect	1st third	2nd third	Last third
Motionless	20	1	***	***	***	***
		2	***	***	***	*
	60	1	***	***	***	***
		2	***	***	***	***
	120	1	**	*	*	n.s.
		2	n.s.			
Take-off	20	1	*	*	n.s.	*
		2	n.s.			
	60	1	n.s.			
		2	n.s.			
	120	1	n.s.			
		2	n.s.			
Bottom-swim	20	1	**	*	***	**
		2	n.s.			
	60	1	**	*	**	n.s.
		2	*	n.s.	*	**
	120	1	n.s.			
		2	n.s.			
Reposition	20	1	**	***	*	n.s.
		2	**	**	n.s.	*
	60	1	***	***	***	***
		2	**	*	***	**
	120	1	n.s.			
		2	n.s.			

60 s trace groups: As in the 20 s trace groups, the number of motionless fish fell during the CS and was lower throughout the CS–US interval (Table 2 and Fig. 6b). Also here the main response was repositions. In contrast to the 20 s trace groups, the occurrence of repositions had no clear peak at the onset of the CS but was more evenly distributed during the CS–US interval (Fig. 6b). The rate was higher than pre-CS level throughout the CS–US interval (Table 2). Bottom-swim occurred at a much lower rate than reposition but more often during the CS–US interval than pre-CS (Table 2 and Fig. 6b). Take-offs were very rare and the rate did not differ from pre-CS level (Table 2 and Fig. 6b).

120 s trace groups: The number of motionless fish decreased slightly after the onset of the CS in Group 1 and was lower than pre-CS level in the first two thirds of the CS–US interval, while there was no difference in Group 2 (Table 2 and Fig. 6b). The rate of repositions was somewhat elevated early in the CS–US interval in Group 1 (Fig. 6c), but the difference did not reach significance (Table 2). Bottom-swim and take-off were rare in both groups (Fig. 6c) and their rates did not differ from the pre-CS levels (Table 2).

3.2.3. Sign-tracking

20 s trace groups: Sign-tracking by moving to the CS was not a major response. The number of fish in the CS sector during the CS was low in both groups and did not differ from pre-CS level in Group 1 ($T_+ = 31.5$, $p > 0.05$), while it was slightly increased in Group 2 ($T_+ = 5$, $p < 0.01$, Fig. 7a). More common than CS approach was orienting towards the CS. The number of fish directed $\pm 45^\circ$ towards the CS light bulb during the CS was higher than pre-CS level (Group 1: $T_+ = 1$, $p < 0.001$; Group 2: $T_+ = 0$, $p < 0.001$, Fig. 7d), although the majority of the fish was not directed towards the CS.

60 s trace groups: The number of fish in the CS sector during the CS did not differ from pre-CS level (Group 1: $T_+ = 40.5$, $p > 0.05$;

Group 2: $T_+ = 23$, $p > 0.05$, Fig. 7b). Similar to the 20 s trace groups, more fish were directed towards the CS bulb during the CS than pre-CS (Group 1: $T_+ = 0$, $p < 0.01$; Group 2: $T_+ = 19.5$, $p < 0.05$, Fig. 7e).

120 s trace groups: The number of fish in the CS sector during the CS did not differ from pre-CS level (Group 1: $T_+ = 2.5$, $p > 0.05$; Group 2: $T_+ = 7.5$, $p > 0.05$, Fig. 7c). Slightly more fish were directed towards the CS bulb during the CS than pre-CS in Group 1 ($T_+ = 2.5$, $p < 0.05$, Fig. 7f). There was no difference in Group 2 ($T_+ = 5$, $p > 0.05$, Fig. 7f).

4. Discussion

This is the first study of anticipatory behaviour in a fish with a “sit-and-wait” foraging strategy. Atlantic halibut are clearly able to associate events separated by at least 60 s. Learning was most rapid during delay conditioning and slowest at the longest trace intervals. The form and temporal distribution of the anticipatory behaviour differed strikingly between delay and trace conditioning, with the fish in the delay procedure swimming towards the surface at the onset of the CS and very few fish remaining on the bottom during the CS–US interval, while in the trace procedure almost no fish left the bottom and at any time in the CS–US interval many fish were lying motionless.

The repositions of halibut in the long-trace procedures were so slight that when we first glanced at the videos we doubted that there were any conditioned responses in the 60 and 120 s trace groups. A preliminary analysis (not presented here), in which the numbers of fish lying motionless 5 s before onset of the CS and 1 s before US release were compared, supported this impression; no clear change from pre-CS to pre-US was detected in the 60 and 120 s trace procedures. However, the more sensitive analyses of all movements throughout the CS–US interval revealed that this was

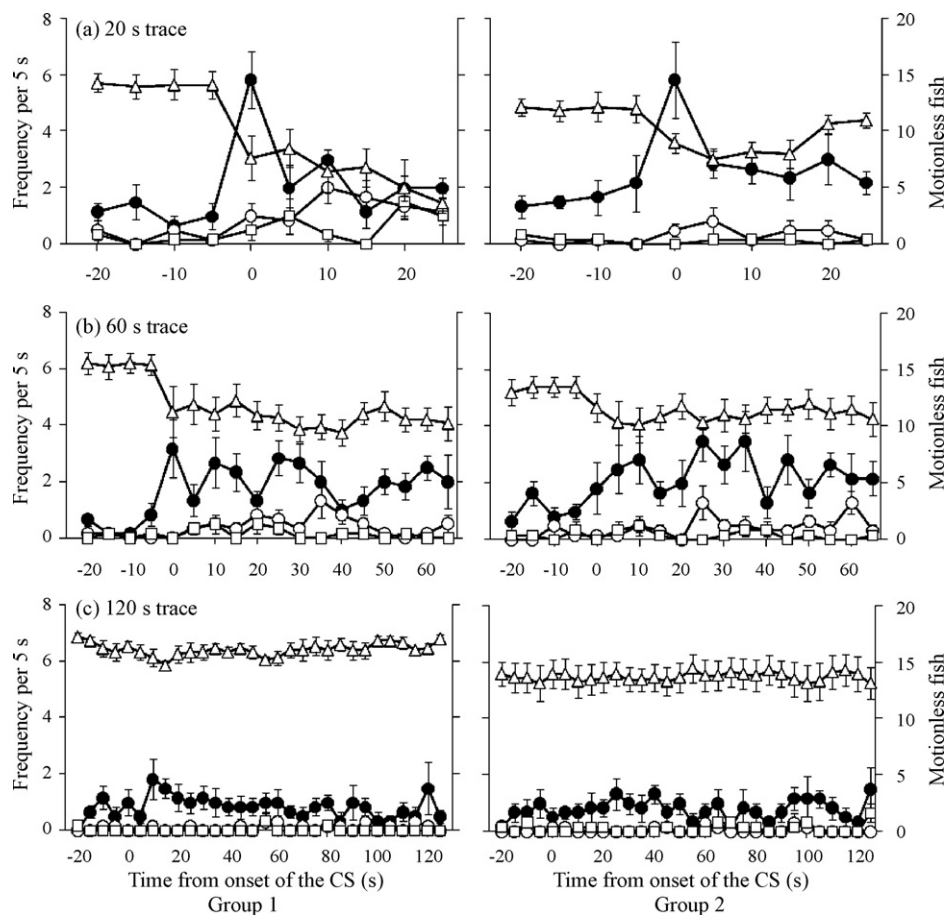


Fig. 6. Behaviour of groups of halibut in relation to time from onset of the CS. Triangles: number of fish lying motionless on the floor at the end of each 5-s interval; filled circles: repositions; open circles: bottom-swims; squares: take-offs. Mean \pm S.E. values based on the final six conditioning trials. (a) The 20 s trace procedure, (b) the 60 s trace procedure and (c) the 120 s trace procedure.

not the case. For both 60 s trace groups the number of fish holding their position was lower during the CS–US interval than during an equally long pre-CS interval, and the magnitude of the difference increased with trial number. A similar pattern was seen in one 120 s trace group. This demonstrates the importance of in-depth analysis of anticipatory behaviour when subtle responses can be expected.

We trained halibut in groups, which might have influenced their behaviour. Since individuals could not be recognized, we do not know how many individuals actually responded to the CS, but the finding that few fish maintained their position throughout the CS–US interval suggests that most fish responded in the delay, 20 and 60 s trace groups. In the 120 s trace group 1, more fish maintained their position, and here only some individuals may have learned. Some individuals may have responded to the behaviour of other individuals rather than to the CS, and social interactions (Brown and Laland, 2006) may have speeded up the learning process. However, halibut is a non-social species (Haug, 1990), and social behaviour presumably had a limited influence on their learning. In any case, social behaviour cannot explain that anticipatory responses were differently expressed in delay and trace procedures, and that learning was slower during conditioning with long trace intervals. Individual halibut may, however, differ in their ability to learn. Kristiansen and Fernö (2007) suggested that feeding motivation of halibut depended on the coping style of the individuals and on how demanding the feeding method was. Longer trace intervals present the halibut with more difficult cognitive situations, and individuals with low coping ability may have changed to a “wait and see” coping strategy (reactive strategy), with fewer of them motivated to learn.

In learning experiments the response rate often increases towards the time of the arrival of rewards (Gallistel and Gibbon, 2000), but this was not found in the present study. Responses were most often initiated early in the CS–US interval (delay and 20 s trace procedures) or had a relatively flat temporal distribution (60 s trace). Also in cod the response peak occurs well before the time of food release (Nilsson et al., 2008a,b). Cod approached the CS (sign-tracked) immediately at its onset, whether the CS was located in the feeding area or on the opposite side of the tank, and regardless of whether they were trained in a delay or trace procedure. Moreover, cod gathered below the feeder waiting for food to arrive throughout a 60 s trace interval (Nilsson et al., 2008a).

Cod are cruising predators that search actively for food and usually pursue or attack prey immediately upon detection (Brawn, 1969; Steingrund and Fernö, 1997). The immediate sign-directed response thus reflects cod feeding strategy. The response of the “sit-and-wait” predator halibut was very different. With the exception of one 20 s trace group, the number of fish near the CS did not increase after the onset of the CS. In contrast to cruising predators, “sit-and-wait” ambush predators often attack prey later and at another location than where it was detected, i.e. when it has entered the lunge range of the predator. This may explain the absence of sign-tracking in halibut. In one delay group, all 20 and 60 s trace groups and one 120 s trace group, the number of fish oriented towards the light bulb rose after the onset of the CS. Cues can thus draw the attention of halibut, in spite of not evoking approach responses. Interestingly, in spite of the significant amount of attention paid to the CS, in all procedures most individuals did not orient themselves directly towards the stimulus. Flatfish eyes are inde-

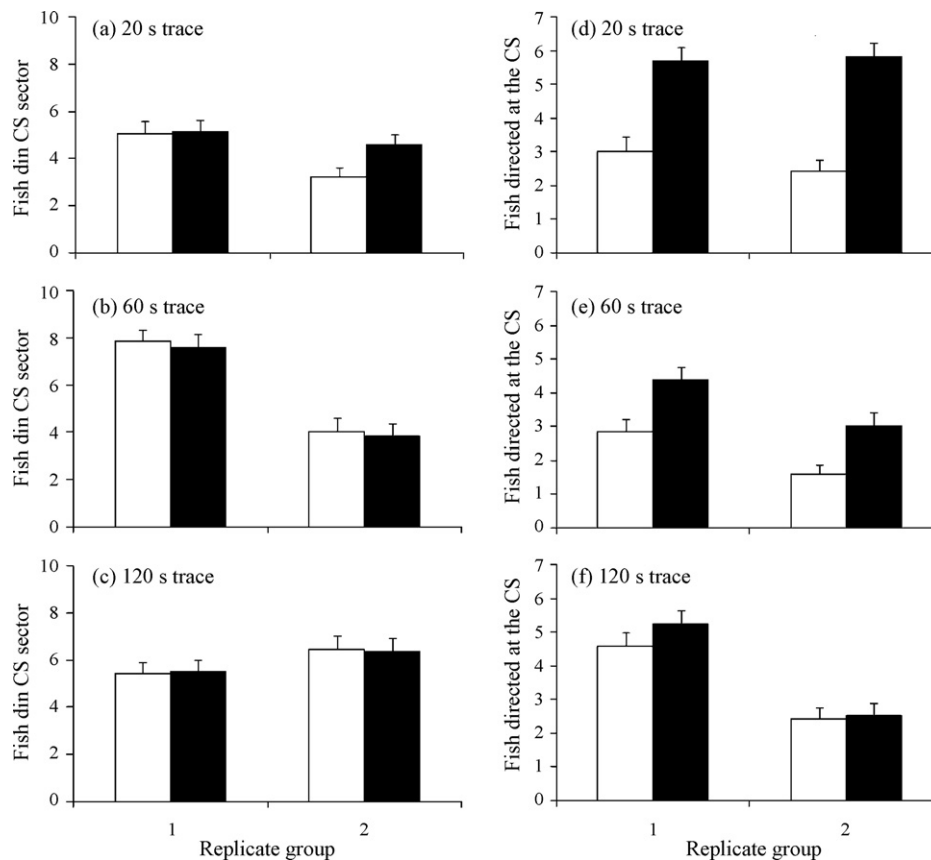


Fig. 7. Sign-directed behaviour of trace conditioned halibut. Open bars: 5 s pre-CS; filled bars: 10 s after the onset of the CS. Number of fish in the CS sector in (a) the 20 s trace procedure, (b) the 60 s trace procedure and (c) the 120 s trace procedure, and number of fish directed $\pm 45^\circ$ towards the CS bulb in (d) the 20 s trace procedure, (e) the 60 s trace procedure and (f) the 120 s trace procedure. Mean \pm S.E. based on all trials on days 10–12 (16 trials for 20 s trace group 1, 17 trials for all other groups).

pendently mobile, giving a 360° angle of vision (Gibson, 2005), and halibut at the floor may have had visual contact with the CS even at an angle of more than 45° .

While approach behaviour towards the CS was generally absent, take-off, i.e. an approach towards the surface, was the main response in the delay procedure. The surface was where food was delivered, and take-off may be seen as a goal-directed behaviour (Boakes, 1977). When the cue temporally overlapped with the reward, goal-directed responses were thus evoked immediately. In contrast, when there was a trace interval between the cue and the reward, few goal-directed approaches were observed, with the response almost exclusively consisting of subtle movements near the floor (repositions and bottom-swims). Furthermore, when the trace interval was long (60 s) these responses had a relatively flat temporal distribution during the CS–US interval. The anticipated time between cue and reward thus has an influence on the form and temporal distribution of the response. The immediate, goal-directed response of the delay conditioned halibut could be seen as attack behaviour. The CS announced that food would be delivered within a few seconds (the CS–US delay was 10 s through most of the experiment), giving the fish little time and little reason to prepare themselves by reorientations on the floor. In contrast, in the trace procedures the CS announced food availability in a more distant future, with enough time to prepare and little reason for immediate approach. In a natural situation with live prey, conspicuous responses might frighten prey not yet within range and thus decrease the chances of successful capture. The differences in response pattern between halibut and cod, with cautious responses in trace conditioned halibut and immediate sign-tracking in cod, appears to be similar to the differences in anticipatory behaviour between the rat and the cat, with increased activity during the

CS–US interval in the former and decreased activity in the latter, in accordance with their respective feeding strategies (van den Bos et al., 2003).

Under natural conditions, the time between cue and encounter will not always be the same, but rather differ from one occasion to another and between different prey types (e.g. free-swimming fish versus bottom-dwelling invertebrates). Where specialized diets are involved, similar responses on all cues may be advantageous. For more diverse diets the time between cues and prey encounters is presumably highly variable, with appropriate form and timing of response difficult to achieve. Predators may either learn to respond differently to different cues, or choose an intermediate response. It would be interesting to train halibut in a procedure with a highly variable CS–US interval or to switch from a period with short-interval trials to long-interval trials and vice versa, in order to see how this species deals with more realistic temporal relationships.

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