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School density affects the strength of collective avoidance responses in wild-caught Atlantic herring *Clupea harengus*: a simulated predator encounter experiment

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An experimental study in a semi-controlled environment was conducted to examine whether school density in wild-caught Atlantic herring *Clupea harengus* affects the strength of their collective escape behaviours. Using acoustics, the anti-predator diving responses of *C. harengus* in two schools that differed in density were quantified by exposing them to a simulated threat. Due to logistical restrictions, the first fish was tested in a low-density school condition (four trials; packing density = 1.5 fish m⁻³; *c.* 60000 fish) followed by fish in a high-density school condition (five trials; packing density = 16 fish m⁻³; *c.* 60 000 fish). The *C. harengus* in a high-density school exhibited stronger collective diving avoidance responses to the simulated predators than fish in the lower-density school. The findings suggest that the density (and thus the internal organization) of a fish school affects the strength of collective anti-predatory responses, and the extent to which information about predation risk is transferred through the *C. harengus* school. Therefore, the results challenge the common notion that information transfer within animal groups may not depend on group size and density.

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Key words: acoustics; anti-predatory behaviour; information transfer; large shoals; pelagic fish; schooling.

INTRODUCTION

Schooling is generally understood as an adaptation that reduces the risk of predation (Seghers, 1981; Magurran, 1986; Pitcher & Parrish, 1993). To date, several mechanisms have been proposed to explain the benefits of schooling in fishes. These mechanisms include increased security through the dilution of risk (Pitcher & Parrish, 1993), improved efficiency in predator detection (Webb, 1980; Magurran *et al.*, 1985), capacity to visually confuse predators (Landeau & Terborgh, 1986; Ioannou *et al.*, 2008) and the ability to perform co-ordinated evasive manoeuvres (Pitcher & Wyche, 1983; Pitcher & Parrish, 1993). In their natural environment, many marine pelagic fishes aggregate in shoals that can span over large spatial scales (km) and can be comprised

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of several millions of individuals (Misund, 1993; Makris *et al.*, 2006). A particularly fascinating property of massive animal aggregations is their collective behaviour, *i.e.* the ability of individuals to react in a synchronous and co-ordinated manner to environmental perturbations or attacks by predators (Couzin *et al.*, 2005; Buhl *et al.*, 2006; Ballerini *et al.*, 2008*a*; Ward *et al.*, 2008; Couzin, 2009; Romanczuk *et al.*, 2009; Yates *et al.*, 2009; Cavagna *et al.*, 2010; Guttal & Couzin, 2010).

Recent research in the field of collective animal behaviour has shifted focus from the importance of global properties (*i.e.* group size) to the local properties (*i.e.* local density or information transfer) as well as considering interindividual rules of interaction to explain how groups form, move and react as a singular unit (Aoki, 1982; Giardina, 2008; Ioannou et al., 2011). Observations of collective phenomena such as co-ordinated escape manoeuvres in large-scale animal aggregations have led to questions regarding how information spreads among individuals in a group (Parrish & Edelstein-Keshet, 1999; Ballerini et al., 2008a; Cavagna et al., 2010). The capacity to rapidly transfer threat-related information across an aggregation regardless of its size is a fundamental component of safety for group-living prey. Schooling fishes can learn about immediate danger through different sensory pathways by gathering visual, hydraulic, acoustic or chemical cues produced directly during the predator's approach or by the evasive behaviours of risk-aware school mates (Chivers & Smith, 1998; Chivers et al., 2001; Dionne & Dodson, 2002; Brown & Magnavacca, 2003; Kim et al., 2009). A significant effort has been directed at quantifying long-range information transfer in co-ordinated animal groups (Buhl et al., 2006; Ballerini et al., 2008b; Cavagna et al., 2010), and particularly in fish schools (Gerlotto et al., 2006; Makris et al., 2009; Marras et al., 2012). The speed at which information spreads through fish schools has the ability to outpace the speed of an approaching predator (Godin & Morgan, 1985; Marras et al., 2012) or the swimming speed of any individual within the school, thus ensuring a rapid propagation of predator cues. Several mechanisms have been proposed to explain the undamped transmission of information within schools over very large distances such as waves of agitation (Radakov, 1973; Axelsen et al., 2001; Gerlotto et al., 2006), fast pressure pulses produced by the startle responses of risk-aware fishes (Gray & Denton, 1991) or compressional density waves (Axelsen et al., 2001; Makris et al., 2009). These cues may act synergistically to structure prey collective responses.

It has recently been suggested that collective responses in animal groups are mediated by the topological distance, *i.e.* the number of companions each group member interacts with, rather than the metric distance that defines a volume around each group member (Ballerini *et al.*, 2008*a*; Cavagna *et al.*, 2010; Niizato & Gunji, 2011). In large wild flocks of starlings *Sturnus vulgaris*, Ballerini *et al.* (2008*a*) found that each bird interacts only with a fixed number of neighbours (around seven), irrespective of how distant those neighbouring birds are. The topological distance notion appears to provide an adequate explanation for how a group regardless of its size may be able to react as a coherent body to environmental perturbations, and in particular how information can flow effectively through a group without being limited by the group size (Cavagna *et al.*, 2010; Bialek *et al.*, 2012). An important assumption of the topological distance hypothesis is that if individuals (*e.g.* schooling fishes) are interacting based on the topological distance, rather than on the metric distance, the emergent collective reactions (*e.g.* escape manoeuvres of a school) would not be influenced by variations in distance between group members and therefore by changes in aggregation (*e.g.* school) density or size (Ballerini *et al.*, 2008*a*; Cavagna *et al.*, 2010, 2013). This can account for the long-range transfer of undamped information throughout large fish schools, which is thought to be advantageous for mitigating the effects of predation. For example, in an estuarine system, predatory spotted sea trout *Cynoscion nebulosus* (Cuvier 1830) were shown to split up prey schools, reducing the behavioural correlations in shoals of Gulf menhaden *Brevoortia patronus* Goode 1878 (Handegard *et al.*, 2012) with possible decreased predator-avoidance efficiency.

In this study, a test was made of whether school density affected the strength of collective diving responses in schooling wild-caught Atlantic herring Clupea harengus L. 1758 by conducting a simulated predator encounter experiment in a semi-controlled environment (sea cage). Clupea harengus collective evasive reactions in two schooling conditions (low and high density) were observed and quantified using acoustics to visualize the diving escape response, a common anti-predator strategy used by wild schooling C. harengus under threat (Pitcher et al., 1996; Nøttestad & Axelsen, 1999; Wilson & Dill, 2002). Under the topological distance hypothesis formulated by Ballerini et al. (2008a), there should be no difference in the escape responses of the high and low-density school. The alternative hypothesis (related to the metric distance), however, is that the closer distance between neighbouring fish in a high-density school could enhance the propagation of threat-related cues among schooling fish through different sensory pathways (e.g. visual, hydraulic, acoustic or chemical) as more fish will interact in a given volume. In this scenario, greater collective evasive reactions in denser schools would be expected compared to lower-density schools. As far as is known, this is the first experimental study on a natural-sized C. harengus school that investigated the effects of school density on collective evasive behaviour.

MATERIALS AND METHODS

The experimental apparatus consisted of two adjacent rectangular sea cages of similar dimensions $[12 \text{ m} \log \times 12 \text{ m} \text{ wide} \times 12 \text{ m} \text{ deep} (Fig. 1)]$ located at the Institute of Marine Research aquaculture facility at Austevoll, Norway (60° 5′ 20″ N; 05° 15′ 58″ E). Prior to the experiment, one of the sea cages, *i.e.* the holding net pen, was stocked with a school of adult C. harengus, c. 60 000 individuals, collected in April 2012 by a commercial purse-seine vessel on the west coast of Norway. After capture, the school was transported and maintained in the holding net pen as described in the study of Rieucau et al. (2014). Total length (L_T) and mass (M) were measured for fish caught using a landing net $[n = 155; L_T = 31.4 \pm 2.2 \text{ cm}; M = 219.3 \pm 50.2 \text{ g};$ index of fish condition, $K(K = 1000 M L_T^{-3}) = 7.02 \pm 0.93$; all results are expressed as mean \pm s.D.]. The experimental tests were conducted during 5-11 July 2012 in the second sea cage (*i.e.* the experimental net pen), which was connected to the holding pen by a net tunnel to allow for transfer of fish from one pen to the other (Fig. 1). The fish were herded into the connecting net tunnel by gradually reducing the depth of the holding net pen using a motorized winch. After the desired quantity of fish was transferred, the net tunnel was closed. The white colour of the net tunnel was found to be aversive to the fish (pers. obs.), possibly due to increased conspicuousness. As a result, all the fish immediately swam to the opposite net pen as soon as the exit side of the net tunnel was opened.

The experiment consisted of two distinct periods during which fish experienced simulated predator encounters (Fig. 2). First, a fraction of the wild-caught school was transferred to the experimental net pen to constitute the low-density school. Second, all the fish remaining in the holding net pen were displaced to the experimental net pen to form the high-density school. The fish were allowed to acclimate to the experimental pen for 12 h following transfer (Fig. 2). Due to logistical restrictions, it was not possible to reverse the density changes (from low to high density and then, from high to low density) during the study as it is a common practise in smaller-scale or



FIG. 1. Schematic representation of the housing and the experimental net pens, and the connecting net tunnel employed during fish transfers. Attacks in the experimental net pen were simulated using a bottle-shaped black predator model. An upward-looking 120 kHz split-beam echosounder (Simrad EK 60) was placed at the bottom of the experimental pen to monitor *Clupea harengus* behaviour.

laboratory experiments. A black-coloured predator model was built from a plastic bottle (34 cm long \times 9 cm wide) covered with water-resistant black vinyl tape. This predator model was highly detectable by schooling *C. harengus*, as it provided visual and hydrodynamic cues (Rieucau *et al.*, 2014). The model was visually conspicuous against the light background when observed from bottom (Weber contrast = -0.089 ± 0.012 ; mean \pm s.D.) and created water displacement when in motion underwater (water displacement velocity = 0.073 ± 0.018 m s⁻¹; mean \pm s.D.) (Rieucau *et al.*, 2014).

To simulate a predator attack, the model was pulled across the pen at 1 m depth by attaching a fishing line to the model and leading it through a block on the opposite end of the experimental net pen (Fig. 1). The end of the fishing line was fastened to an elastic shock cord that was extended to constant length and attached to a fixed point located 20 m from the net pen. The



FIG. 2. Experimental procedure timeline presenting the date of each trial for the low-density *Clupea harengus* school condition (four trials; packing density = 1.5 fish m⁻³; *c*. 6000 fish) and the high-density *C. harengus* school condition (five trials, packing density = 16.1 fish m⁻³; *c*. 60 000 fish). Each trial consisted of two experimental treatments (predator model and control) separated by a 6 min interval and presented in random order. First, the low-density school was tested from 5 July to 8 July 2012. On 8 July 2012, fish were transferred from the housing net pen to the experimental net pen, after the last trial of the low-density school condition, to constitute the high-density school. The high-density school was tested from 9 July to 11 2012.

motion of the model through the school was induced by releasing the fishing line and allowing the tension of the elastic shock cord to propel the model through the net pen. The speed of the predator model was measured by timing the tow duration which was consistent among trials $(3.40 \pm 0.41 \text{ m s}^{-1}; \text{ mean} \pm \text{ s.p.})$. Because of their drag differences, the speed of the control fishing line was matched up to the speed of the predator model by manually towing the fishing line along the same path.

All behavioural tests were conducted in the experimental net pen. To measure the strength of the diving responses, a calibrated upward-looking 120 kHz split-beam echosounder with a 7° beamwidth (Simrad EK 60; www.simrad.com) mounted on a gimbal close to the bottom of the experimental net pen was employed. The behaviour of *Clupea harengus* was continuously monitored using acoustics and a high-resolution underwater camera located at 10 m depth (image sensor Sony Super HAD CCD; www.sony.com) before and during the experimental periods. The data collected were imported into Echoview 5.2 (SonarData Pty. Ltd; www.echoview.com), and the extent of the avoidance reactions to the experimental treatments was manually quantified by measuring the vertical dimensions of the diving responses in echograms (Fig. 3).

The main objective of this study was to compare collective patterns in *C. harengus* schools that differed only in density and thus in their internal structure. The collective diving responses of fish were therefore examined only when they presented polarized, aligned and circular swimming patterns (ascertained using an underwater camera; Fig. 4), in accordance with Pitcher & Parrish's (1993) definition of schooling. For each density condition, fish experienced a series (four for the low-density condition and five for the high-density condition) of two experimental treatments (predator model and control) presented in random order, for a total of 18 exposures (Fig. 2). The control treatment consisted of the fishing line without a model and was used to test whether the noise from the releasing gear, activity on the dock and the motion of the fishing line itself may have caused the fish evasive reactions. The predator model and control measurements were interspersed with other tests in a randomized block design (Rieucau *et al.*, 2014; Handegard *et al.*, in press). A 6 min interval was established between two exposures within an experimental series to allow the fish to return to a similar schooling dynamic as prior to exposure and exposures to the predator model were separated by at least 5 h.

The primary aim was to investigate whether school density affected the collective response of *C. harengus* when the school sizes matched the social conditions that fish experienced in their natural environments. Therefore, the logistical restriction occurred was not possible to create several smaller sub-sets to control for pseudoreplication (Hurlbert, 1984). Due to the large number of fish in both density conditions and their highly dynamic swimming pattern, however, it is likely that this has created a substantial mixing of individuals ensuring that not always the same fish directly encountered the predator model or the fishing line for each release. Given that the low-density school was tested first, a larger response of the high-density school compared to the low-density school cannot be attributed to habituation alone. In addition, the randomized order in which stimuli were presented and the interval of time between consecutive exposures may have prevented habituation and a sequence effect in responsiveness in the *C. harengus* as suggested by Schleidt *et al.* (1983).

STATISTICAL ANALYSIS

A paired t-test was used to compare an estimate of school density, the volume backscattering coefficient (s_v expressed in m⁻¹) (Maclennan *et al.*, 2002), 1 min before and 3 min after exposure for both school sizes. First, the average S_v estimates (*i.e.* volume backscattering strength in the logarithmic domain expressed in dB rel 1 m⁻¹) was obtained, which provides a measure proportional to the logarithm of fish density, in Echoview, and then the s_v was calculated following: $s_v = 10^{(0.1s_v)}$ (Maclennan *et al.*, 2002). To quantify the density differences between the two experimental schools, the s_v values were compared 1 min before the exposure of the experimental treatments with a single factor analysis of variance (ANOVA). The density of *C. harengus* (*i.e.* fish m⁻³) within the acoustic beam was estimated as $s_v \sigma_{bs}^{-1}$, where σ_{bs} is the backscattering cross section of a 31.4 cm fish at 6 m depth based on the relationship described for *C. harengus*, *i.e.* $\sigma_{bs,120\text{kHz}} = \sigma_{bs,38\text{kHz}} \times 0.50$ (Saunders *et al.*, 2012), to convert the 38 kHz estimates of σ_{bs} to 120 kHz.



0 Time relative to stimulus start (s)

50

100

150

FIG. 3. Examples of echograms illustrating the strength of vertical collective responses of Clupea harengus in (a) the high-density school (16.1 fish m⁻³) and (b) the low-density school (1.5 fish m⁻³) during exposure to the predator model. Also presented is a time series of the volume backscattering strength (S_v), expressed in dB re 1 m⁻¹, relative to the start of the experimental treatment.

-50

-100

-150



FIG. 4. Underwater photographs of (a) the high-density (16·1 fish m⁻³) *Clupea harengus* school and (b) the low-density school (1·5 fish m⁻³) before the exposure the experimental treatment.

Whether the vertical distribution of *C. harengus* prior to exposure differed between the two density conditions was tested using a one-way ANOVA. The distribution of *C. harengus* in the water column was quantified by manually measuring the vertical dimension of the school in echograms. A one-way ANOVA was used to investigate the effect of the experimental treatments on the strength of the diving responses as a function of school density. All analyses were conducted in Statistica 11 (StatSoft, Inc.; www.statsoft.com). The control data (fishing line alone) were not included in the statistical analysis as it never induced vertical avoidance responses from the school in either density condition.

RESULTS

The estimated densities of the high and low-density *C. harengus* schools were $S_v = -26.81 \pm 0.47 \,\text{dB}$ re $1 \,\text{m}^{-1}$ and $S_v = -37.02 \pm 0.52 \,\text{dB}$ re $1 \,\text{m}^{-1}$ (mean \pm s.D.), respectively. No significant difference in S_v before and after exposure was detected for the high-density school (n = 10, t = -0.274, d.f. = 9, P > 0.05) or the low-density school (n = 8, t = -0.922, d.f. = 7, P > 0.05), which suggests that, after 3 min, the school returned a similar packing density and orientation (as backscatter depends on both densities and fish orientation; Foote, 1985) as prior to the exposure. The s_v values 1 min before exposure to the experimental treatments showed that the densities of the two schools were significantly different ($F_{1.16} = 556.72, P < 0.001$; Fig. 5), with a



Low-density school High-density school

FIG. 5. Acoustic density estimates represented by the volume backscattering coefficient (s_v) (mean + s.b.), 1 min before (**■**) and 3 min after exposure (**■**) to the two experimental treatments (control + predator model) for the high (16·1 *Clupea harengus* m⁻³) and low-density (1·5 fish m⁻³) school conditions.

ratio of 0.095 between the low and the high-density schools. This s_v ratio indicates that the density of fish in the acoustic beam was c. 10 times higher in the high-density school than in the low-density school. This corresponds to an approximate density of 1.5 fish m⁻³ in the echosounder beam in the low-density school, and 16.1 fish m⁻³ in the high-density school.

There was no difference in the vertical extent of fish in the water column prior to exposure to the experimental treatments between the low-density and the high-density conditions ($F_{1,16} = 3.01$, P > 0.05). This indicates that the two schools differed in density as they occupied approximately the same volume of the net pen prior to the stimuli (Fig. 6). The strength of the diving responses, expressed as the vertical extent of disturbance, differed significantly between the high and low-density conditions ($F_{1,7} = 11.12$, P = 0.01) (Fig. 6) with significantly stronger diving responses being observed in the dense school condition (Table I).

DISCUSSION

Using acoustics, a high-density *C. harengus* school was found to exhibit stronger diving responses to a simulated predator than a lower-density school. Although the two schools differed by a factor of 10 in number of individuals, they occupied the same volume in the water column before being exposed to the experimental treatments. This indicates that the two schools differed primarily in density and ultimately in their internal structure, *i.e.* the spatial distance between neighbouring fish. An increase in the distance among school members was observed to reduce the strength of collective evasive responses in *C. harengus* schools, which is not consistent with the topological distance hypothesis.

Natural variations in school density have been reported (Nøttestad *et al.*, 1996) and are thought to reflect changes in fish states and fitness trade-offs (*e.g.* feeding, survival or reproduction). Several studies showed that hungrier fishes spend less time in larger



FIG. 6. Maximum depth of the collective responses (mean + s.D.) to the predator model (■) and *Clupea harengus* school vertical extent (*i.e.* distance of the lower school boundary from the surface) before exposure (■) for the high (16·1 fish m⁻³) and low-density (1·5 fish m⁻³) school conditions. The figure shows stronger collective diving responses for the high-density school when exposed to the predator model. The two schools did not differ in their depth distribution.

groups than well-fed fishes (Barber & Huntingford, 1995; Reebs & Saulnier, 1997). In addition, non-feeding pelagic fish shoals are generally larger than feeding shoals (Nøttestad *et al.*, 1996). A mechanistic explanation is that variations in shoal size and density can be induced by simple individual behavioural rules based on attraction and repulsion towards nearby fishes (Katz *et al.*, 2011) in combination with attraction to external stimuli such as food resources. Recently, these behavioural rules have been found to remain constant across shoal sizes (Katz *et al.*, 2011; Gautrais *et al.*, 2012; Tunstrøm *et al.*, 2013) and have been used to explain how fishes in shoals swim in an co-ordinated manner and respond to the movement of their neighbours (Herbert-Read *et al.*, 2011; Katz *et al.*, 2011). Moreover, attraction and repulsion can be modulated by hunger state and predation risk. In feeding fishes, or those strongly motivated to feed, the relative attraction to conspecifics is expected to decrease resulting in more

TABLE I. Vertical extent of *Clupea harengus* schools and school density estimates (s_v) prior to stimuli exposure (control + predator model) for the low-density (1.5 fish m⁻³) and high-density (16.1 fish m⁻³) schools. Also shown is the strength (m) of the collective vertical responses for the predator model treatment in the two density conditions

	$\frac{\text{School vertical distribution}}{F_{116} = 3.01, P > 0.05}$		$\frac{s_{\rm v} ({\rm m}^{-1}) \text{ before exposure}}{F_{1,16} = 556 \cdot 72, P < 0.001}$		$\frac{\text{Collective response}}{F_{1,7} = 11 \cdot 12, P = 0 \cdot 01}$	
Density conditions	Mean	S.D.	Mean	S.D.	Mean	S.D.
Low-density school	6.84	0.16	0.0002	0.000024	2.76	0.29
High-density school	7.21	0.14	0.0021	0.00022	4.09	0.26

individualistic behaviour with increasing dispersion as food level decreases (Robinson & Pitcher, 1989; Sogard & Olla, 1997; Hensor *et al.*, 2003). Conversely, non-feeding fishes, with no other force influencing them other than the risk of predation, will exhibit less individualistic behaviour and form denser shoals as predation risk increases (Fréon *et al.*, 1992; Gerlotto *et al.*, 2006). In comparison, Holmin (2013) estimated the density of a wild *C. harengus* school in the Norwegian Sea outside the feeding and reproduction periods to be $S_v = -33.2 \text{ dB}$ re 1 m⁻¹. Then, it is reasonable to assume that the social conditions that fish experienced in the high-density school may reflect those of *C. harengus* in the wild during periods where the prime motivation is survival rather than reproduction or feeding, while the low-density school may correspond to school densities observed during periods in which the fitness trade-offs may have shifted towards feeding.

The present observations suggest that information transfer of predator cues in a fish school is affected by the density of the school, and therefore by the spatial distance between fish. This raises additional questions about how collective fish behaviours emerge, and in particular how information propagates throughout large fish schools. In this study, diving responses were compared among schools where the fish presented polarized and circular swimming patterns as well as high levels of alignment (Fig. 3). Polarization and alignment are considered as key features of collective motion in schooling fishes as they promote rapid propagation of information through schools (Herbert-Read *et al.*, 2011) and reduce the energetic costs of locomotion and risk of collision (Partridge & Pitcher, 1980). Marras *et al.* (2012) found that after being threat-ened by an artificial stimulus, schooling *C. harengus* remained aligned or regained their pre-exposure degree of alignment rapidly (<1 s), which highlights the importance of aligned swimming for schooling fish. The present findings, however, suggest that distances between individuals may also play an important role in structuring the collective behaviour of schooling fishes under the risk of predation.

Acquiring reliable quantitative measurements of behaviour of aquatic organisms in natural environments is a challenging task. The use of acoustics has proved to offer a unique opportunity to describe and quantify structural characteristics and the dynamic behaviour of fish shoals (Pitcher *et al.*, 1996; Simmonds & MacLennan, 2005; Holmin, 2013). This technique also allows for investigations of fine-scale predator–prey interactions in the field (Handegard *et al.*, 2012). In natural conditions, acoustic data can be used to measure the characteristics of wild marine fish schools, including density, shape and size (Misund, 1993).

Acoustics is also well suited for tracking the behaviour of schools during stimuli-response studies (Axelsen *et al.*, 2001; Nøttestad *et al.*, 2002; Handegard & Tjøstheim, 2005; Doksæter *et al.*, 2012; Peña *et al.*, 2013), and has been employed to detect the onset of schooling in very large fish aggregations (Makris *et al.*, 2009). By applying this technique in a controlled environment, it was demonstrated that the density of a wild-caught *C. harengus* school affects the collective anti-predatory responses, which can ultimately have important effects on the outcome of predator–prey interactions.

One intriguing result is that only the shallowest part of the school reacted to the simulated threat in both density conditions. The observed differences in diving responses to the near-surface predator model may reflect decreased information transfer with increasing depth. A possible explanation is that the extent to which information spreads through the school may depend on the initial number of fish that responded to the experimental stimulus (Domenici & Batty, 1994, 1997; Marras *et al.*, 2012). If the mechanism underlying information transfer in fish aggregations is topological, reactions should propagate further in low-density groups as the information has to pass through fewer individuals for the same distance compared to high-density groups. As stronger collective reactions in the high-density school were observed, it is unlikely that the response could be explained solely by the topological mechanism. At this point, further empirical studies investigating the mechanisms at work during the transfer of threat information among schooling fishes and the potential role of school internal structure are warranted.

During fishery or aquaculture activities, fish manipulation using net gears can induce important behavioural, physiological (e.g. anoxia) and physical impairments (e.g. scale loss and skin injuries) increasing stress levels as well as delayed mortality as shown in Sardina pilchardus (Walbaum 1792) (Marcalo et al., 2008, 2010, 2013) and C. harengus (Tenningen et al., 2012). Among the reported behavioural effects caused by stressful manipulations, drastic changes in schooling tendency (e.g. reduced swimming speed, increased inter-fish distances, reduced school cohesion, incapability to maintain circular and co-ordinated swimming patterns) and lower responsiveness to predators resulting in a greater vulnerability to predation have been found in S. pilchardus (Marcalo et al., 2013). Despite there being no physiological measurements made on the fish (e.g. cortisol, lactate or blood ions levels; Tenningen et al., 2012), it is likely that the resting time after the transfer was sufficient for C. harengus to recover from the stress induced by transferring fish from the housing net pen to the experimental net pen as <12 h after the transfer, fish that formed the high-density school, returned to highly polarized, co-ordinated and circular swimming pattern. Moreover, the stronger evasive reactions found in the high-density school, which followed the transfer, compared to the low-density school suggest that the transfer procedure did not have disrupted or altered the schooling tendency, internal organization and responsiveness to simulated threats of captive C. harengus. In an experiment investigating stress and mortality effects associated to purse seining in C. harengus, Tenningen et al. (2012) found that mortality after short periods of time at crowding densities $<150 \text{ kg m}^{-3}$ (corresponding c. to 680 fish m⁻³ in this study) was negligible. It is likely that the crowding density during the transfer procedure in the experiment remained below 680 fish m⁻³ and the acoustically estimated packing densities for the low-density and high-density schools were much lower with 1.5 and 16.1 fish m⁻³, respectively. In addition, no increase in mortality after the transfer and after the experiment (daily monitoring conducted by the fish keepers at the Institute of Marine Research aquaculture facility) was noticed, suggesting that C. harengus tolerated the handling.

The confinement of the *C. harengus* in the experimental net pen may have influenced or restricted diving responses compared to an open system. It is unlikely, however, that this confinement led to the observed differences in strength in diving responses between the two density conditions. In particular, space limitation cannot explain why a smaller proportion of fish reacted to the presented stimuli when in a low-density school compared to when in a larger and denser school. Moreover, given that the low-density school was tested first, a larger response of the high-density school compared to the low-density school cannot be attributed to habituation alone.

To corroborate the results, future *in situ* studies that focus on the role of school density on the common anti-predator diving strategy used by wild *C. harengus* to escape predators are required. Nevertheless, this study provides useful insight that points to the need for further examination of the rules of interaction that determine how fish in schools move together, make collective decisions and respond to each other.

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