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# BRIEF COMMUNICATIONS

## A grid-net technique for the analysis of fish positions within free-ranging shoals

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The design and operation of a grid-net are described, which allows the capture of entire shoals of wild fish and preserves information about the two-dimensional structure of shoals and the spatial positions of individual fish within the shoal. This simple technique facilitates investigation of several aspects of individual differences in fish shoaling behaviour in the wild for the first time.
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Key words: sampling device; social group; spatial position; shoaling.

Fish shoals have proven to be a good study system for the investigation of individual differences within social groups. Theoretical work (Hamilton, 1971; Eggers, 1976; Barta *et al.*, 1997) has been largely supported by laboratory studies demonstrating that the costs and benefits of grouping are not equally distributed across spatial positions within the group. Heterogeneity between positions in both feeding benefits and predation risks has been reported for a number of species; individuals in the front part of a shoal have higher feeding rates and consume higher quality food items than fish in other positions (O'Connell, 1972; Major, 1978; Krause *et al.*, 1992, 1998*a*), although front and peripheral shoal positions may be more exposed to predation than rear or central positions (Bumann *et al.*, 1997; Krause *et al.*, 1998*b*).

Laboratory studies have suggested that individual fish may vary in their tendency to occupy particular shoal positions depending on individual traits such as size (Krause *et al.*, 1998*a*), hunger (Krause *et al.*, 1998*a*) and parasite load (Barber & Huntingford, 1996). However, to date field studies demonstrating positional differences within free-ranging shoals are scarce, largely because of the practical difficulties in examining individuals with respect to their shoal positions. Techniques previously employed in the field include direct observations and video recording (Krause, 1993) and incomplete sampling of a single shoal (DeBlois & Rose, 1996). Here a simple grid-net design is presented, which allows entire wild fish shoals to be collected in shallow water while preserving information on the relative position of individuals.

The grid-net consists of a flat 50 × 50 cm frame of light stainless steel mesh, forming a grid of 25 × 25 mm squares, overlaid with a continuous section of fine (1 mm mesh) nylon netting. The net is pushed through each square in the frame to produce 5 cm deep pockets of netting with open tops in a 20 × 20 grid beneath the frame [Fig. 1(a)]. Pockets are sewn along the top edges to attach them permanently to the frame and prevent bunching of the material. Four steel rods are attached along the sides of the frame for support, and marked with the *x* and *y* coordinates as grid reference points for each pocket. The frame and netting material are darkened to match the substratum using

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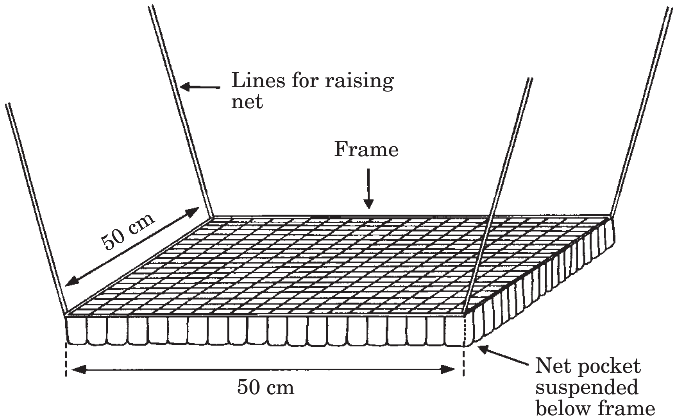
(a)




(b)

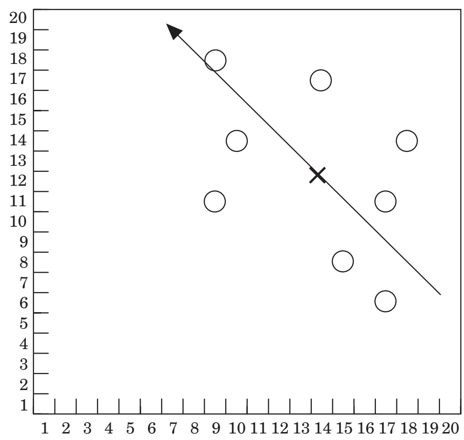



FIG. 1. (a) Schematic diagram of the grid-net design. Each square in the grid opens into a single pocket, shown unfolded below the frame. (b) Results of a typical school capture. Individual fish positions (○) are recored as the $x$, $y$ coordinates for each pocket. The direction of movement (estimated for the whole shoal) is depicted as an arrow through the centroid ( × ).

permanent markers and fabric dye. The net is positioned with the frame lying flat on the substratum, the net pockets folding flat beneath the frame. Non-elastic strings attached to the four corners of the frame allow the net to be lifted smoothly by two observers when a school passes over the net. Individual fish are thus captured in separate pockets such that the ‘ grid references ’ of fish produce a record of two-dimensional positioning within the shoal and also provide information on the two-dimensional structure of the entire shoal [Fig. 1(b)].

Control trials were conducted in a 2 × 2 m laboratory tank to assess the accuracy of the technique and estimate the error in determining relative positioning of fish using this method. The grid-net was placed centrally in the tank in 20 cm of water before introducing fish in each trial. Juvenile banded killifish *Fundulus diaphanus* (Lesueur) were collected by seine from Morice Lake, New Brunswick, Canada, where the species is common in small shoals in the shallow littoral zone (Krause *et al.*, 2000*a*). A total of 61 fish (mean ± S.D. total length, $L_T$=28·4 ± 5·8 mm) were assigned to groups ranging in size from two to ten fish, matched by $L_T$ ( ± 3 mm) within groups, and individual groups were introduced into the experimental tank where they generally formed single shoals exhibiting swimming behaviour very similar to that seen in the field. Trials were recorded using a Sony DSR-PD100AP digital video camera mounted above the tank, and the net was raised by two observers from behind a blind. Once fish had initiated shoaling and the entire shoal passed directly over the frame, the net was lifted by pulling swiftly on the lines to raise the frame in a single smooth motion. The key to successful capture using this technique was the initial rapid motion of the frame to enclose fish in the pockets, after which the net could be gradually brought to the surface for processing without any escape of fish. Rapid raising of the net is possible because the design is such that only the light frame of the grid needs to be drawn through the water during this phase, while the pockets themselves unfold without the need to shift a large volume of water. This aspect of the design is facilitated in larger models by increasing pocket depth and adding weights to the bottom of each pocket.

An estimate of the mean direction of travel of the shoal needs to be recorded to allow the definition of front and rear positions within a shoal after capture. This was done by visually tracking the shoal before capture and placing a metre ruler onto the frame immediately after capture, orientated along the axis of travel. The front and rear coordinates of the shoal axis were then recorded from the grid [Fig. 1(b)]. The direction of travel of individual fish was subsequently measured from video in the frame before capture as a vector running through the head and dorsal fin of the fish (which were aligned in the direction of travel even when the posterior part of the body was curved in movement), and this trajectory was confirmed by reference to preceding frames. These measurements were then used to assess the accuracy of the estimated shoal direction.

The results of grid-net captures such as this can subsequently be analysed with regard to front and rear positioning (Krause *et al.*, 1992), or peripheral and central positioning (Bumann *et al.*, 1997), with the centroid of the school defined as the mean *x*, *y* co-ordinates.

Fifteen shoals containing a total of 61 fish were recorded in laboratory trials. Mean ± S.D. catch duration (time from first movement of net to fish entering pockets) was 0·19 ± 0·03 s. Nearest-neighbour distances (*NND*) for each fish were measured from video in the frame before capture ($NND_1$) and compared with postcapture measurements taken from the centre of pockets in which fish were caught ($NND_2$), providing an error-estimate of how much individual fish moved relative to their neighbours during capture. This includes both active movement by fish during capture, and displacement of fish relative to each other as a result of entering the net, which is a function of pocket width.

As distances between fish within a shoal are not truly independent because fish may respond to shoalmates other than their nearest neighbour (Partridge, 1981), analyses were conducted on shoal means ($n$=15). For shoal means, median nearest-neighbour distance before capture ($NND_1$) was 2·2 (quartiles 1·6, 2·5) $L_T$, and 2·1 (quartiles 1·4, 2·3) $L_T$ after capture ($NND_2$). There was no significant difference between shoal mean *NNDs* before and after capture (Wilcoxon paired-sample test $z$=0·398, $n$=15, $P$=0·69), suggesting that *NNDs* were not consistently larger or smaller following capture.

Individual fish were tracked through successive frames on video to determine their movements during capture. Error in *NND* for individuals were calculated as the absolute difference between $NND_1$ and $NND_2$ expressed as a percentage of $NND_1$. The shoal mean of this value was then calculated. Across all shoals the median error was 25·2% (quartiles 10%, 46·5%), indicating that although individual fish were shifted slightly during capture in the grid-net, the distances by which they were displaced are small relative to the natural distances between fish, and not sufficient to cause two fish to exchange their original positions within the shoal. The shoal mean percentage error in *NND* was not related to shoal size (Spearman rank correlation: $r$=0·34, $n$=15, $P$=0·22), or to the shoal mean of $L_T$ ($r$=0·37, $n$=15, $P$=0·17).

To examine the effects of the grid-net on shoal shape, shoal circumference was measured from video in the frame before captured ($C_1$) and compared with that derived from postcapture co-ordinates ($C_2$); the shoal circumference was defined as the smallest convex polygon which included all fish, and was applied to the nine groups which contained more than two fish. Fish which form the vertices of this polygon are defined as being on the periphery of the shoal. The median error in shoal circumference ($C_1 - C_2$ as a percentage of $C_1$) was 7·8% (lower quartile 3·0%; upper quartile 15·3%), and there was no significant difference between $C_1$ and $C_2$ (Wilcoxon paired-sample test $z = -1{\cdot}48$, $n = 9$, $P = 0{\cdot}14$). There was no relationship between per cent circumference error and shoal size ($r = 0{\cdot}16$, $n = 15$, $P = 0{\cdot}69$).

To examine the accuracy of the estimates of shoal direction, the angular direction of each fish was determined from video in the frame before capture, and converted into unit vectors. The mean vector for each shoal was then calculated and compared with the unit vector derived from the visual estimate of shoal direction. The median amount by which the estimated direction deviated from the shoal mean direction was 6·5° (lower quartile 3·7°; upper quartile 19·9°). Overall, the estimated shoal direction was not significantly different from the calculated shoal mean vector (Wilcoxon matched pairs test $z = -0{\cdot}65$, $n = 15$, $P = 0{\cdot}51$). The median amount by which individual fish deviated from the shoal mean vector was 7·6° (lower quartile 3·3°; upper quartile 15·7°), indicating that shoals were quite strongly polarized. The magnitude of individual deviations from the estimated shoal direction was very similar (median = 7·5°; lower quartile 3·5°; upper quartile 19·5°), suggesting that this estimate provided a good approximation of shoal polarization. It should be noted that the future position of the shoal may be better predicted from the heading of the leading fish (Krause *et al.*, 2000*b*), but the shoal mean vector provides an accurate axis of front and rear positions for all fish in the shoal at any one instant in time.

The assignment of shoal positions from the grid-net coordinates were compared with positions accurately determined from video footage (relative positions of fish in the frame before capture). Fish were defined as being in the front half of a shoal if they were in position 1 to $n/2$ for shoals of even size and 1 to $(n-1)/2$ for shoals of odd size (Krause *et al.*, 1992). Two fish were excluded from this analysis as they were captured in the same pocket and thus their positions could not be resolved. Of 56 fish to which positions were assigned using the grid-net, four (7%) were ‘ wrongly ’ assigned to front and rear positions (i.e. assigned to one half of the shoal from video footage, and the other half of the shoal from the grid-net). Subsequent examination of video footage showed that all four of these fish were in the same shoal, and their assignment to incorrect positions arose from them moving substantially as the net was lifted, rather than to errors in estimating shoal direction. Similarly, fish were assigned to peripheral or central shoal positions with an error of 7% (two of 28 fish), although the sample size was small for this control. It is concluded that incorrect assignment of individual positions is rare using this technique, and could potentially be reduced further by close attention to the shoal during lifting so that samples in which fish scatter over the net can be discarded from the analysis.

Overall the control measurements indicate that error in assessing relative positions between fish (arising either through active movement of fish or passive displacement due to the grid) is low using this technique, supporting the potential of the grid-net design for providing important field data on within-shoal differences in individual spatial positioning.

In field trials of the grid-net, conducted in the littoral zone of Morice Lake in September 2000 (Ward *et al.*, in press), free-ranging killifish shoals were easily caught as they passed over the net. The technique was the same as that described for laboratory trials, except that the net was positioned in a shallow depression in the sandy substratum such that the edges of the frame were level with the surrounding area.

The technique as described here was developed specifically for shoals of small freshwater fish in shallow water, but the general design may potentially be applied to other systems, for example by using pocketed nets on a larger scale in deeper water. For large nets, the use of anchored floats attached to the upper edge of the frame are recommended to ensure rapid raising of the net as used with ‘ popnet ’ designs (Morgan *et al.*, 1988). The efficacy of grid-net designs for other fish species depends upon a number of factors including fish swimming speed, shoal size and fish size, which should be given careful consideration before data collection. Pocket size should be chosen to closely match fish size and typical *NNDs* for the species under investigation, so that pockets are small enough to retain detailed information on shoal positions but large enough to avoid unduly displacing fish from natural positions during lifting. In the present study small shoals (<10 fish) were used to demonstrate the efficacy of the technique. Shoals containing large numbers of fish will require a larger grid than that described here, but should in principle operate in the same manner. Most importantly, three-dimensional shoals in deeper water cannot be analysed with respect to peripheral and central positions, as peripheral fish in the top or bottom of the shoal will be captured in central pockets. Analysis should be restricted to front and rear positions for such shoals. Although grid-nets provide information on shoal structure in only two dimensions, the use of alternative techniques such as multiple cameras to record three-dimensional positions is technically challenging in the field, and two dimensional resolution of positioning can reveal important features of shoal structure including body length and parasitism (Ward *et al*., in press), initiation of swimming directions (Krause *et al.*, 1998*a*), feeding rates (Krause, 1993) and predation risk (Bumann *et al.*, 1997).

Grid-nets are particularly appropriate for the collection of shoal positioning data currently unobtainable from alternative techniques such as video recordings of wild shoals. Such data includes accurate body length measurements, mass and body condition, age, sex, stomach contents, parasite infection status and even individual identities (in conjunction with marking or manipulation procedures; Krause, 1993). The simple grid-net design described here makes possible the collection of such data from free-ranging schools in the field for the first time.

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