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The effects of parasitism and body length on positioning within wild fish shoals

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Summary

1. The influence of body length and parasitism on the positioning behaviour of individuals in wild fish shoals was investigated by a novel means of capturing entire shoals of the banded killifish (*Fundulus diaphanus*, Lesueur) using a grid-net that maintained the two-dimensional positions of individuals within shoals.
2. Fish in the front section of a shoal were larger than those in the rear.
3. Individuals parasitized by the digenean trematode (*Crassiphiala bulboglossa*, Haitsma) showed a tendency to occupy the front of shoals. Parasitized fish were also found more in peripheral positions than central ones in a significant number of shoals.
4. Shoal geometry was affected by the overall parasite prevalence of shoal members; shoals with high parasite prevalence displayed increasingly phallanx-like shoal formations, whereas shoals with low prevalence were more elliptical.
5. There was no relationship between body length and parasite abundance or prevalence in the fish population which suggests body length and parasite status are independent predictors of positioning behaviour.
6. Solitary individuals found outside shoals were both more likely to be parasitized and had higher parasite abundance than individuals engaged in shoaling.
7. Differences in the shoaling behaviour of parasitized and unparasitized fish are discussed in the context of the adaptive manipulation hypothesis.

Key-words: body length, geometry, parasitism, positioning, shoaling.

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Introduction

Fish shoals present biologists with an ideal opportunity to investigate social living and self-organization in vertebrates. Despite the attention paid to these subjects, relatively little is known about fish shoals in the wild and still less is known about the positioning of individuals in such shoals. The absence of a method for capturing entire free-ranging shoals while preserving the intrashoal positional integrity has until now prevented the testing of laboratory predictions in the field.

The composition of a fish shoal affects its members in a number of ways, particularly in terms of individual predation risk and foraging efficiency (see Krause *et al.* 2000 for a review). These costs and benefits vary for different positions throughout the shoal. A number of studies have reported increased foraging success for individuals in the front of a shoal. DeBlois & Rose (1996) reported that individuals foraging at the front of a large moving shoal of Atlantic cod (*Gadus morhua*) gained more food items and those food items tended to be of higher quality than those gathered by fish at the rear of shoals. Major (1978) recorded biased foraging success of leading fish in shoals of striped jack (*Caranx ignobilis*). However, there is an associated cost with occupying front positions in a moving group in terms of increased per capita risk of predation. Bumann, Krause & Rubenstein (1997) modelled predator encounters with moving prey groups and were able to identify a significantly greater predation risk to individuals at the front of groups. These findings were supported by empirical data (Krause, Ruxton & Rubenstein 1998). In both studies, lead fish were attacked and killed by a predator significantly more than fish towards the rear.

There are a number of phenotypic and state-dependent factors known to influence the positioning behaviour of individuals. In a laboratory study (Krause, Reeves & Hoare 1998), found that large fish occupied the front positions in shoals more frequently than small conspecifics. Differences in swimming speed between small and large fish was proposed as a mechanism for this.

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The nutritional state of an individual is also known to affect its position. Krause, Bumann & Todt (1992) demonstrated an initial preference of experimentally food-deprived roach (*Rutilus rutilus*) to occupy front positions when joining a shoal of conspecifics. These individuals subsequently enjoyed higher feeding rates. However, once the experimentally food-deprived individuals satisfied their nutritional requirements their preference for the front of the shoal disappeared.

Laboratory studies on the positioning behaviour of parasitized individuals have also revealed significant trends. Parasitized fish were more likely to occupy peripheral shoal positions and have greater nearest neighbour distances than unparasitized fish [Krause & Godin 1996: banded killifish (*Fundulus diaphanus*); Barber & Huntingford (1996): minnow (*Phoxinus phoxinus*)]. Both studies investigated parasites with complex life-cycles which use fish as intermediate hosts. It has been suggested that there should be high selection pressure in such cases for parasites to evolve strategies to manipulate their intermediate host to increase the probability of transmission to their final host, often a piscivorous bird (Poulin 2000). This argument is known as the adaptive manipulation hypothesis and has received support from a number of empirical studies (e.g. Lafferty & Morris 1996). The mechanism by which the parasitic worms brought about the above changes in fish behaviour is not known but could potentially include starving or disorientating the host.

This study investigates the shoaling behaviour of the banded killifish (*F. diaphanus*) in the field with specific consideration of the influence of body length and of parasitism on intrashoal positioning and shoal geometry. The banded killifish is an intermediate host of the trematode parasite, *Crassiphiala bulboglossa.* Infected fish develop an externally visible, pigmented cyst which is not horizontally transmissible (Olsen 1974). The exclusive final host of *C. bulboglossa* is the belted kingfisher, *Megaceryle alcyon*.

Based on previous studies conducted in the laboratory, we predict that:

1. Larger fish should occupy positions towards the front of shoals given that body length and maximum swimming speed are positively correlated (Beamish 1978).

2. Parasitized individuals will be more likely to occupy positions at the front and in the periphery of shoals or to occur outside shoals as solitary individuals.

3. Moving shoals should exhibit an ellipsoid geometry with shoals being longer than they are wide along the axis of locomotion as this minimizes the predation risks associated with shoal leadership (Bumann *et al*. 1997).

General methods

STUDY SITE

The study site was an 80-m strip of the littoral zone of a northern bay of Morice Lake (near Sackville, New Brunswick, Canada) (45°55′ N, 64°21′ W). The northern shore of the lake where this study was conducted is not accessible to the general public and is fringed by trees to the west. A combination of these factors and the presence of high fish densities provides suitable habitat for the belted kingfisher and hence the trematode parasite, *C. bulboglossa*. In fact, belted kingfishers have been observed nesting in the vicinity (personal observation). The lake bed shelved gently (ratio approximately 1 : 20), the substrate was fine sand with aquatic vegetation being absent at the study area. The water was clear.

PROCEDURE

Entire free-ranging shoals were collected using a grid-net, which holds individual fish within pockets of netting. The design and operation of the grid-net is described in detail in Hoare *et al.* in press). The grid-net was situated in 15 cm of water, 2·5 m from the shore. An area of the lake bed equal to that of the grid-net (0·25 m^2) was excavated to form a shallow (1 cm) recess to accommodate the grid-net, ensuring that it lay flush with the surrounding substrate. Once the net was in place, a small amount of the excavated sand was distributed about its surface to act as additional camouflage. When an entire shoal crossed over the grid-net it was lifted rapidly and synchronously by two observers using cords fastened to the net. If it could be established clearly by both observers that the entire shoal had been captured, the grid-net pocket position, total length (mm) and parasite abundance of each individual fish was recorded. In addition, the overall shoal direction of travel was noted with respect to the grid-net.

In a further study, single fish (defined as being > 10 body lengths from a conspecific) were captured using the grid-net. This method allowed us to confirm that no other fish were in the vicinity and therefore that the fish captured were indeed solitary. Total length and parasite status were recorded for all single fish. Furthermore, a random sample of the banded killifish population at the site used for the grid-net was taken in order to provide background information on the parasite status of the local killifish population as a whole using a 10-m fine (5 mm) mesh seine net. All fish captured were measured, inspected for parasites and returned.

The banded killifish was selected for this study as it is one of the most common species in Morice Lake (Godin & Morgan 1985) and forms two-dimensional shoals in the littoral zone.

DATA ANALYSIS

Two-dimensional positions of individual fish within a shoal were recorded as x, y coordinates on the grid-net. Laboratory controls were conducted using this technique to estimate the intrinsic error (see Hoare *et al.* in press).

The centroid was calculated for each shoal. To divide shoals into front and rear sections a line was drawn perpendicular to the direction of travel and bisecting the centroid. Fish in front of this line were deemed to be in the front of the shoal, those behind the line were deemed to be in the back. Median body length, median parasite abundance and the parasite prevalence were calculated and compared between front and back of each shoal. Distance from the centroid was also determined for each individual fish and comparisons were made between median distance from the centroid for parasitized and unparasitized individuals. The latter comparison was made only in shoals containing more than two fish and where both parasitized and unparasitized fish were present.

The degree of the elongation of each shoal was measured by treating shoal members as data points and carrying out a linear regression. The angle between the regression line and the shoal direction of travel was determined as shown in Fig. 1. Comparisons were made regarding this angle for shoals composed of > 50% parasitized fish and for shoals composed of < 50% parasitized fish.

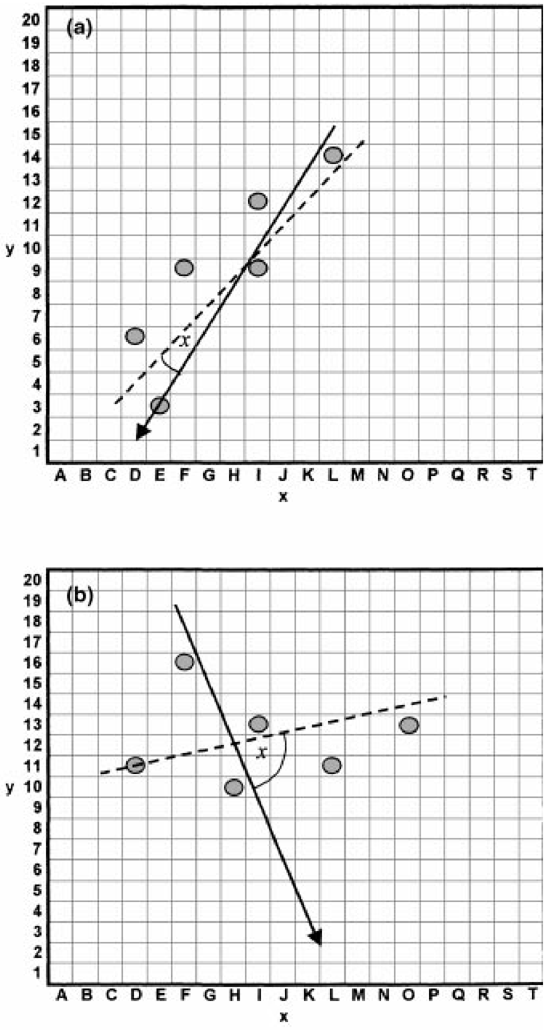

Fig. 1. Illustration of variability in shoal geometry. (a) Shows small angle x between direction of travel (→) and linear regression trendline (---) denoting a characteristically processional shoal. (b) Shows a large angle between direction of travel and a linear regression trendline characteristic of a phallanx-type shoal formation. Circular filled symbols indicate positions of shoal members recorded on the grid-net.

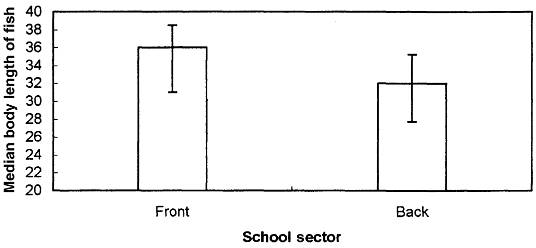

Fig. 2. Comparison of median body lengths between fish in the front and rear of a shoal. Error bars show quartiles. $N = 21$ shoals.

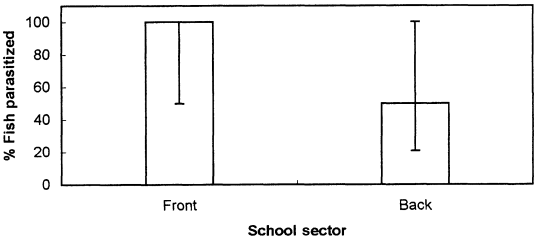

Fig. 3. Comparison of parasite prevalence between fish in the front and rear of a shoal. Error bars show quartiles. $N = 21$.

Results

A total of 21 complete shoals comprising 128 fish were captured using the grid-net. Shoal size ranged from two individuals to 24, with a median shoal size of five (lower quartile = 3; upper quartile = 8). The median body length for all individuals captured using the grid-net was 34 mm (lower quartile = 29 mm; upper quartile = 38 mm) and parasite prevalence was 62%. There was no correlation between body length and parasite prevalence (binary logistic regression: $r = 0{\cdot}03$, $P = 0{\cdot}14$, $N = 128$) or parasite abundance (Spearman's rank: $r_s = 0{\cdot}135$, $P = 0{\cdot}13$, $N = 128$).

Median body length and parasite prevalence were greater in the front of a shoal than in the rear (Wilcoxon matched pairs test: body length $Z = 2{\cdot}66$, $P = 0{\cdot}008$, $N = 21$ (Fig. 2); parasite prevalence $Z = 2{\cdot}55$, $P = 0{\cdot}011$, $N = 21$ (Fig. 3)). However, parasitized fish in the front of shoals did not have greater parasite abundance than parasitized fish to the rear (Wilcoxon matched pairs test: $Z = 0{\cdot}36$, $P = 0{\cdot}71$, $N = 16$).

Parasitized fish were not significantly further from the shoal centroid than unparasitized fish (Wilcoxon signed ranks test: $Z = 1{\cdot}22$, $P = 0{\cdot}11$ $N = 14$). However, this was due to the presence of an outlier. Parasitized fish were further from the shoal centroid than unparasitized fish in 11 of the 14 shoals (binomial test: $P = 0{\cdot}029$).

Shoals composed of a majority of parasitized fish showed greater angles between a fitted linear regression trendline and the direction of travel than shoals composed of a majority of unparasitized fish (Mann–Whitney *U*-test: $Z_{3,12} = 2{\cdot}6$, $P = 0{\cdot}009$, $N = 15$) (see Fig. 1).

A total of 18 solitary (non-shoaling) killifish were captured. The parasite abundance and prevalence of these fish were compared with that of 18 shoaling individuals which were drawn randomly from the 128 fish caught using the grid-net. The solitary fish were more likely to be parasitized than fish from a random sample (resampling test: $P = 0{\cdot}048$, $N = 1000$) and showed higher parasite abundance (resampling test: $P = 0{\cdot}005$, $N = 1000$).

Discussion

This study is the first to document the influence of parasitism on positioning behaviour of individuals and to report differences in body length between fish at the front and back of entire free-ranging shoals. Fish in the front half of a shoal were more likely to be parasitized than their shoal-mates at the back of a shoal. They were not, however, likely to have greater parasite abundance. Furthermore, we found that individuals at the front of shoals were larger than their conspecifics in the back of the shoal.

Trends within shoals concerning body length may be explained by variation in competitive ability, feeding motivation and swimming ability. Foraging rewards are greater for the fish at the front of moving shoals and stronger competitors should occupy front positions especially in environments with low predation regimes. The greater swimming capability of large fish (Beamish 1978) in terms of speed and stamina also seems likely to be a contributory factor, but not enough is yet known about the swimming speeds of shoaling fish.

Individuals in the front of a shoal were significantly more likely to be parasitized than individuals in the rear of shoals. By exploiting the hosts energy reserves, *C. bulboglossa* appears to manipulate host behaviour (Barber, Hoare & Krause 2000), increasing its motivation to feed and causing the infected killifish to seek shoal positions that will maximize its foraging rate, such as in the front or on the periphery of shoals. However, we did not find that individuals in the front of a shoal were more heavily parasitized than the fish in the rear. This suggests that while parasites may manipulate positioning behaviour by increasing foraging motivation, more heavily parasitized individuals may be unable to either withstand the foraging competition associated with shoaling or are unable to shoal for other reasons, such as increased swimming costs. The cyst may deleteriously affect the hydrodynamic efficiency of the parasitized individual. Similarly, the positioning of the cyst on or around the dorsal musculature may impinge on swimming ability by obstructing blood flow, precipitating atrophication of the muscle or otherwise disrupting motor function. Krause & Godin (1996) reported a lack of motor control in heavily parasitized fish. This means that parasitized individuals are likely to have to work harder than unparasitized fish, or individuals with lower parasite abundance, to maintain a position towards the front of a shoal. It is also likely that heavily infected individuals need to spend more time foraging to attain the same nutritional benefit as marginally or non-parasitized individuals (Barber *et al.* 2000). This may explain the observed trend for higher parasite abundance observed in the solitary fish caught. The isolation of an individual of a shoaling species is likely to increase its likelihood of being predated, which is potential evidence for the phenomenon described by Lafferty (1999) as 'parasite increased trophic transmission' (PITT). Lafferty & Morris 1996 were able to show that killifish parasitized by trematode worms suffered greater predation, increasing the probability of transmission of the parasite to its final host.

It is unknown whether differences in positioning behaviour (i.e. more time spent in front and peripheral positions) makes parasitized fish more prone to kingfisher predation because no data have yet been collected on the success of kingfisher attacks on different shoal positions. It is likely, however, that a putative reduction in vigilance associated with incresaed feeding requirements, and the isolation of heavily parasitized individuals, could both contribute to an increased predation risk.

The difference in shoal geometry between shoals consisting mainly of parasitized individuals and shoals made up mainly of unparasitized individuals may be explained by heterogeneity in foraging benefits between shoal positions and the hypothesized higher feeding motivation of parasitized fish. As a position on the leading edge of a moving shoal is the most beneficial for individuals with high metabolic requirements, it appears intuitive that a shoal of parasitized fish might arrange themselves such that the entire shoal becomes like a leading edge, i.e. phallanx-shaped, to maximize foraging benefits. Shoals containing a majority of unparasitized individuals with lower foraging requirements may be expected to adopt a more risk-aware strategy with respect to shoal positions. This could impinge on shoal geometry by producing more typically aligned and elongated shoals.

Useful further work would include additional data on the role played by passive factors such as swimming speed in shaping shoal positions. More information needs to be gathered on the existence of any increased incidence of predation by both the definitive host and by non-hosts on parasitized fish (Lafferty & Morris 1996).

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References

Able, K.W. (1990) Life history patterns of New Jersey salt marsh killifishes. *Bulletin of the New Jersey Academy of Science*, **35**, 23–20.

Arme, C. & Owen, R.W. (1967) Infections of the three-spined sticklebacks, *Gasterosteus aculeatus* L. with the plerocercoid larvae of *Schistocephalus solidus* (Muller 1776) with special reference to pathological effects. *Parasitology*, **57**, 301–314.

Barber, I., Hoare, D.J. & Krause, J. (2000) The effects of parasites on fish behaviour: an evolutionary perspective and review. *Reviews in Fish Biology and Fisheries*, **10**, 1–35.

Barber, I. & Huntingford, F.A. (1996) Parasite infection alters schooling behaviour: deviant positioning of helminth-infected minnows in conspecific groups. *Proceedings of the Royal Society of London, Series B*, **263**, 1095–1102.

Beamish, F.W.H. (1978) Swimming capacity. *Fish Physiology*, vol. vii (eds W.S. Hoar & D.J. Randall), pp. 101–187. Academic Press, New York.

Bumann, D., Krause, J. & Rubenstein, D. (1997) Mortality risk of spatial positions in animal groups: the danger of being in the front. *Behaviour*, **134**, 1063–1074.

DeBlois, E.M. & Rose, G.A. (1996) Cross-shoal variability in the feeding habits of migrating Atlantic cod (*Gadus morhua*). *Oecologia*, **108**, 192–196.

Godin, J.-G., J. & Morgan, M.J. (1985) Predator avoidance and shoal size in a cyprinodontid fish, the banded killifish (*Fundulus diaphanus*, Lesueur). *Behavioural Ecology and Sociobiology*, **16**, 105–110.

Hoare, D.J., Ward, A.J.W., Couzin, I.D., Croft, D.P. & Krause, J. (in press) A grid-net technique for the analysis of fish positions within free-ranging fish shoals. *Journal of Fish Biology*.

Krause, J., Bumann, D. & Todt, D. (1992) Relationship between position preference and nutritional state of individuals in schools of juvenile roach (*Rutilus rutilus*). *Behavioural Ecology and Sociobiology*, **30**, 177–180.

Krause, J. & Godin, J.G.J. (1996) Influence of parasitism on shoal choice in the banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae). *Ethology*, **102**, 40–49.

Krause, J., Hoare, D.J., Croft, D.P., Lawrence, J. & Ward, A.J.W. & Ruxton, G.D. (2000) Fish shoal composition: mechanisms and constraints. *Proceedings of the Royal Society of London, Series B*, **267**, 2011–2017.

Krause, J., Reeves, P. & Hoare, D.J. (1998) Positioning behaviour in roach shoals: the role of body length and nutritional state. *Behaviour*, **135**, 1031–1039.

Krause, J., Ruxton, G.D. & Rubenstein, D.I. (1998) Is there an influence of group size on hunting success? *Journal of Fish Biology*, **52**, 494–501.

Lafferty, K.D. (1999) The evolution of trophic transmission. *Parasitology Today*, **15**, 111–115.

Lafferty, K.D. & Morris, A.K. (1996) Altered behaviour of parasitised killifish increases susceptibility to predation by bird hosts. *Ecology*, **77**, 1390–1397.

Major, P. (1978) Predator–prey interactions in two schooling fishes, *Caranx ignobilis* and *Stolephorus purpureus*. *Animal Behavior*, **26**, 760–777.

Olsen, O.W. (1974) *Animal Parasites: their life cycles and ecology*. University Park Press, Baltimore.

Poulin, R. (2000) Manipulation of host behaviour by parasites: a weakening paradigm? *Proceedings of the Royal Society London, Series B*, **267**, 787–792.

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