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

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Running headline: WARD et al: POSITIONING IN WILD FISH SHOALS

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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 (<u>Fundulus</u> <u>diaphanus</u>) 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 parasitised by the digenean trematode (<u>Crassiphalia</u> <u>bulboglossa</u>) showed a tendency to occupy the front of shoals. Parasitised 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 load 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 parasitised and had higher parasite loads than individuals engaged in shoaling.

7. Differences in the shoaling behaviour of parasitised and unparasitised fish are discussed in the context of the adaptive manipulation hypothesis.

Fish shoals present biologists with an ideal opportunity to investigate social living and self-organisation 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 whilst preserving the intra-shoal 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. O'Connell (1972) found lower food capture rates of rear individuals in shoals of the northern anchovy (Engraulis mordax). 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 et al. (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 et al. 1998a). 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 context-dependent factors known to influence the positioning behaviour of individuals. In a laboratory study, Krause et al.

(1998b) 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.

The nutritional state of an individual is also known to affect its position. Romey (1995) studied the positioning behaviour of whirligig beetles (Gyrinus sp.) in relation to their nutritional state. Individuals were removed from groups and deprived of food. When those individuals were reintroduced to groups they showed a preference for positioning themselves on the periphery of the group where they were able to gather the most food items. Krause et al. (1993) 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.

Studies on the positioning behaviour of parasitised individuals have also revealed significant trends. Parasitised fish were more likely to occupy peripheral shoal positions and have greater nearest neighbour distances than unparasitised fish (Krause & Godin: 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 the host. Starvation would be an effective way of making fish take up

peripheral shoal positions where food intakes are higher or to make them leave a shoal

entirely thus increasing risk of predation and transmission to the final host (Lafferty

1999).

This study investigates the shoaling behaviour of the banded killish (F. diaphanus) with specific consideration of the influence of body length and of parasitism on intra-shoal positioning and shoal geometry. The banded killifish is an intermediate host of the trematode parasite, Crassiphalia bulboglossa. Infected fish develop an externally visible, pigmented cyst which is not horizontally transmissable (Hoffman 1956; 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 swimming speed are positively correlated (Beamish 1978).

2. Parasitised 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 (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

1   fringed by trees to the west. A combination of these factors and the presence of high

2   fish densities provides suitable habitat for the belted kingfisher and hence the

3   trematode parasite, C. bulboglossa. In fact, belted kingfishers have been observed

4   nesting in the vicinity (pers. obs.). The lake bed shelved gently (ratio approximately

5   1:20), the substrate was principally fine sand with aquatic vegetation being absent in

6   the vicinity of the study area. The water was clear.

7

8   Procedure.

9   Entire free-ranging shoals were collected using a grid-net, which holds individual fish

10   within pockets of netting. The design and operation of the grid net is described in

11   detail in Hoare et al. (see enclosed copy). The grid net was situated in 15 cm of water,

12   2.5 m from the shore. An area of the lake bed equal to that of the grid net (0.25 m^2)

13   was excavated to form a shallow (1 cm) recess to accommodate the grid net, ensuring

14   that it lay flush with the surrounding substrate. Once the net was in place, a small

15   amount of the excavated sand was distributed about its surface to act as additional

16   camouflage. When an entire shoal crossed over the grid net it was lifted rapidly and

17   synchronously by two observers using cords fastened to the net. If it could be clearly

18   established by both observers that the entire shoal had been captured, the grid net

19   pocket position, total length (mm) and parasite load of each individual fish was

20   recorded. In addition, the overall shoal direction of travel was noted with respect to

21   the grid net.

22   In a further study, single fish (defined as being > 10 body lengths from a

23   conspecific) were captured using the grid net. This method allowed us to confirm that

24   no other fish were in the vicinity and therefore that the fish captured were indeed

25   solitary. Total length and parasite status were recorded for all single fish. Furthermore,

1 a random sample of the banded killifish population at the site used for the grid-net

2 was taken in order to provide background information on the parasite status of the

3 local killifish population as a whole using a 10 m fine (5 mm) mesh seine net. All fish

4 captured were measured, inspected for parasites and returned.

5 The banded killifish was selected for this study as it is one of the most

6 common species in Morice Lake (Godin & Morgan 1985) and forms two dimensional

7 shoals in the littoral zone.

8

9 <u>Data Analysis</u>

10 Two-dimensional positions of individual fish within a shoal were recorded as x, y co-

11 ordinates on the grid-net. Laboratory controls were conducted using this technique to

12 estimate the intrinsic error. These are discussed in Hoare <u>et al.</u> (see enclosed copy).

13 The centroid was calculated for each shoal. To divide shoals into front and rear

14 sections a line was drawn perpendicular to the direction of travel and bisecting the

15 centroid. Fish in front of this line were deemed to be in the front of the shoal, those

16 behind the line were deemed to be in the back. Median body length, median parasite

17 load and the parasite prevalence were calculated and compared between front and

18 back of each shoal. Distance from the centroid was also determined for each

19 individual fish and comparisons were made between median distance from the

20 centroid for parasitised and unparasitised individuals. The latter comparison was only

21 made in shoals containing more than two fish and where both parasitised and

22 unparasitised fish were present.

23 The degree of the elongation of each shoal was measured by treating shoal

24 members as data points and plotting a linear regression. The angle between the

25 regression line and the shoal direction of travel was determined as shown in Fig. 1.

1   Comparisons were made regarding this angle for shoals composed of $> 50\%$

2   parasitised fish and for shoals composed of $< 50\%$ parasitised fish.

3

4   RESULTS

5

6   A total of 21 complete shoals comprising 128 fish were captured using the grid net.

7   Shoal size ranged from 2 individuals to 24, with a median shoal size of 5 (lower

8   quartile = 3; upper quartile = 8). The median body length for all individuals captured

9   using the grid net was 34 mm (lower quartile = 29 mm; upper quartile = 38 mm) and

10   parasite prevalence was 62%. There was no correlation between body length and

11   parasite prevalence (Spearman Rank: $\underline{r}_s = 0.132$, $\underline{P} = 0.14$, $\underline{N} = 128$) or parasite load

12   (Spearman Rank: $\underline{r}_s = 0.135$, $\underline{P} = 0.13$, $\underline{N} = 128$).

13   Median body length and parasite prevalence were greater in the front of a shoal

14   than in the rear (Wilcoxon matched pairs test: body length $\underline{Z} = 2.66$, $\underline{P} = 0.008$, $\underline{N}=21$

15   (Fig. 2); parasite prevalence $\underline{Z} = 2.55$, $\underline{P} = 0.011$, $\underline{N} = 21$ (Fig. 3)). However,

16   parasitised fish in the front of shoals did not have greater parasite loads than

17   parasitised fish to the rear (Wilcoxon matched pairs test: $\underline{Z} = 0.36$, $\underline{P} = 0.71$, $\underline{N} = 16$) .

18   Parasitised fish were not further from the shoal centroid than unparasitised fish

19   when tested using a matched pairs test (Wilcoxon signed ranks test: $\underline{Z} = 1.22$, $\underline{P} = 0.11$

20    $\underline{N} = 14$). However, this may have been due to both the small sample size and the

21   presence of outliers. Parasitised fish were further from the shoal centroid than

22   unparasitised fish in 11 of the 14 shoals (Binomial Test: $\underline{P} = 0.029$).

23   Shoals composed of a majority of parasitised fish showed greater angles

24   between a fitted linear regression trendline and the direction of travel than shoals

1   composed of a majority of unparasitised fish (Mann-Whitney U-test: $\underline{Z}_{3,12}$ = 2.6, $\underline{P}$ =

2   0.009, N = 15) (see Fig. 1).

3   A total of 18 solitary (non-shoaling) killifish were captured. These were

4   compared with the shoaling individuals caught using the grid net. The solitary fish

5   were more likely to be parasitised than fish from a random sample (Resampling test: $\underline{P}$

6   = 0.048, $\underline{N}$ = 1000) and showed higher parasite loads (Resampling test: $\underline{P}$ = 0.005, $\underline{N}$ =

7   1000).

8

9   DISCUSSION

10

11   This study is the first to document the influence of parasitism on positioning within

12   wild fish shoals. It is also the first to report clear body length related front-back trends

13   in entire free-ranging shoals. Fish in the front half of a shoal were more likely to be

14   parasitised than their shoal-mates. They were not, however, likely to have greater

15   parasite loads. Furthermore, we found that individuals at the front of shoals were

16   larger than their conspecifics in the back of the shoal.

17   Trends within shoals concerning body length may be explained by variation in

18   competitive ability, feeding motivation and swimming ability. Large fish have a

19   higher mass-specific metabolic efficiency than do smaller conspecifics (Wootton

20   1994) and may be less motivated to feed. However, as foraging rewards are greater for

21   the fish at the front of moving shoals, stronger competitors should occupy front

22   positions especially in environments with low predation regimes. The greater

23   swimming capability of large fish (Beamish 1978) in terms of speed and stamina

24   seems likely to be a contributory factor, but not enough is yet known about the

25   swimming speeds of shoaling fish.

1   Individuals in the front of a shoal were significantly more likely to be

2   parasitised than individuals in the rear of shoals. By exploiting the hosts energy

3   reserves, C. bulboglossa appears to manipulate host behaviour (Barber et al 2000),

4   increasing its motivation to feed and causing the infected killifish to seek shoal

5   positions that will maximise its foraging rate, such as in the front or on the periphery

6   of shoals. However, we did not find that individuals in the front of a shoal were more

7   heavily parasitised than the fish in the rear. This suggests that whilst parasites may

8   manipulate positioning behaviour by increasing foraging motivation, more heavily

9   parasitised individuals may be unable to either withstand the foraging competition

10   associated with shoaling or are unable to shoal for other reasons, such as increased

11   swimming costs. The cyst may deleteriously affect the hydrodynamic efficiency of the

12   parasitised individual. Similarly, the positioning of the cyst on or around the dorsal

13   musculature may impinge on swimming ability by obstructing blood flow,

14   precipitating atrophication of the muscle or otherwise disrupting motor function.

15   Krause & Godin (1994) reported a lack of motor control in heavily parasitised fish.

16   This means that parasitised individuals are likely to have to work harder than

17   unparasitised fish, or individuals with lower parasite loads, to maintain a position

18   towards the front of a shoal. It is also likely that heavily infected individuals need to

19   spend more time foraging to attain the same nutritional benefit as marginally or non-

20   parasitised individuals (Barber et al. 2000). This may explain the observed trend for

21   higher parasite loads observed in the solitary fish caught. The isolation of an

22   individual of a shoaling species is likely to increase its likelihood of being predated,

23   which is potential evidence for the phenomenon described by Lafferty (1999) as

24   'parasite increased trophic transmission' (PITT). Lafferty & Morris (1996) were able

to show that killifish parasitised by trematode worms suffered increased predation, increasing the probability of transmission of the parasite to its final host.

For the strategy of the parasite to be described as adaptive, it must cause greater predation by the final host on the intermediate host. No data as yet exists to describe the per capita predation risks associated with different positions in shoals of banded killifish in direct relation to predation by the belted kingfisher. However, it is likely that a putative reduction in vigilance associated with increased feeding requirements, and the isolation of heavily parasitised individuals, could both contribute to an increased predation risk.

The difference in shoal geometry between shoals consisting mainly of parasitised individuals and shoals made up mainly of unparasitised individuals may be explained by heterogeneity in foraging benefits between shoal positions and the hypothesised higher feeding motivation of parasitised 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 parasitised fish might arrange themselves such that the entire shoal becomes like a leading edge, i.e. phallanx-shaped, to maximise foraging benefits. Shoals containing a majority of unparasitised individuals with lower foraging requirements may be expected to adopt a more risk-aware strategy with respect to shoal positions adopted. 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 parasitised fish (Lafferty & Morris 1996).

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1   *Figure 1: Illustration of variability in shoal geometry. Fig 3a shows small angle x*

2   *between direction of travel (——) and linear regression trendline (——) denoting*

3   *a characteristically processional shoal. Fig 3b shows a large angle between direction*

4   *of travel and a linear regression trendline characteristic of a phallanx-type shoal*

5   *formation. Circular filled symbols (⬤) indicate positions of shoal members recorded*

6   *on the grid-net.*

7

8   *Figure 2: Comparison of median body lengths between fish in the front and rear of a*

9   *shoal. Error bars show quartiles. N = 21 shoals.*

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11   *Figure 3: Comparison of parasite prevalence between fish in the front and rear of a*

12   *shoal. Error bars show quartiles. N = 21.*

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Median Body Length of Fish

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