Report for Friday Harbor Laboratories Fish Swimming Course 2019

Happy together: Schooling confers hydrodynamic and behavioural advantages in a labriform swimmer (*Cymatogaster aggregata*).

Søs Riber, Huili Xu, Eleanor Gee

Abstract

Fish can enjoy a number of behavioural and hydrodynamic advantages during schooling. Shiner perches, labriform swimmers using primarily pectoral fins and assisting with the caudal fin, were used to study the differences of fin beat frequencies and oxygen consumption between a schooling pair of fish, a solitary fish, and a false pair where a single fish swam alongside a video of a conspecific. By having a fish swimming alongside a video of a swimming fish, compared with the pair of swimming fish, the hydrodynamic effect could be separated from the behavioral/visual effect. It was found that pectoral fin beat frequencies were significantly higher for schools of two-real-fish swimming at 5.5 Bl/s relative to other groups.

Additionally the relationship between oxygen consumption and swimming speed was significantly different between treatments, with the false pair treatment having lower oxygen consumption from 3.5 Bl/s and onwards. Our results suggest that the advantages of schooling are not only hydrodynamic but also behavioural, and that the relative importance of behavioural effects depends on the level of swimming activity.

Key words: Pectoral fin swimming, oxygen, fin beat frequency, hydrodynamics, schooling, false pair, labriform fishes, gait transition.

Introduction

Fish swimming modes can be classified into two generic categories based on their locomotion features: one is body and caudal fin swimming (BCF), and the other is median and paired fin swimming (MPF) (Webb 1984). MPF swimmers can also be divided into two motion types based on their fins movement: drag based and lift based swimming. Labriform swimming, one style of MPF modes commonly employed by many fish species, is associated with lift-based pectoral fin locomotion within a wide range of swimming speeds (Walker and Westneat 1997). However, labriform swimmers use caudal fins at high speeds and burst swimming too. This pectoral-caudal fin transition of labriform fish is defined as gait transition (Drucker and Jensen 1996), which allows them to use BCF motion for more powerful propulsion.

Fish schooling can provide many benefits for fish, such as saving energy, defending against predators and increasing foraging success (Weihs 1973, Johansen et al. 2010, Marras et al. 2015). While in a school, labriform swimmers may utilize the vortices created by leading individuals to enjoy significant energetic benefits (Johansen et al. 2010).

To better understand the hydrodynamic advantages of fish schooling, a number of studies have been performed investigating interactions between fish and oxygen consumption under different conditions. Liao et al. (2003) found that the cost of fish locomotion can be reduced

by using vortices. Weihs (1973) proposed a ‘diamond’ configuration theory of schooling indicating that fish can save energy by taking advantage of adjacent propulsive wakes, but it was pointed out later that some fish do not swim in this pattern (Partridge and Pitcher 1979), which may be due to potential trade-offs in fish schooling (Abrahams and Colgan 1987). In fact, there have been suggestions that every individual in a fish school could reduce swimming cost compared to solitary swimming at the same speed (Marras et al. 2015). In the aspect of energetic benefits, studies have been conducted comparing total oxygen consumption of fish schools with that of individual fish (Ross et al. 1992; Johansen et al. 2010).

It was hypothesized that the company of a fish would help a real swimming fish increase its maximum speed. In addition to study on the hydrodynamic effect of real fish schools, the influence of behavioural effects has been less studied (Parker et al. 1973; Klyashtorin et al. 1980; Lefrançois et al. 2009; Nadler et al. 2016). We specifically hypothesized that in addition to providing hydrodynamic advantages, swimming in a school is associated with reduced stress compared to a lone individual, that:

- increases the maximum swimming speed;
- reduces the cost of transport at a given speed;

Shiner perch *Cymatogaster aggregata*, usually found in loose schools, were used in the present work to investigate the kinematic and energetic differences of fish schooling with a visible fake fish and a real fish. The oxygen consumption and pectoral fin beat frequency of the above two fish school trials were evaluated and compared with a reference group of solitary swimming fish.

Materials and methods

Experimental fish

36 shiner surfperch (*Cymatogaster aggregata*) with a body mass of 20.4 +/- 2.7g, and standard length (9.7 +/- 0.5cm), height (3.1 +/- 0.3cm) and width (1.1 +/- 0.1cm) were collected with beach seines at Jackson Beach (48º31’; 123º01’ W), San Juan Island, Washington, USA, in July and August 2019. Post capture the fish were transferred to Friday Harbor Laboratories, where they were held in 90cm x 60cm x 15 cm tanks, which were continuously supplied with flow-through seawater (salinity 34 ppm) at a temperature of average 13 °C (range from 11°C to 15°C). Fish were transferred to fasting tanks for at least 24 hours prior to an experimental trial, as the postabsorptive state ensures maximal energy for swimming.

Setup

Experiments were conducted in a Steffensen-type swimming tunnel of 5.17 L volume (see Methling et al. 2011 for a design schematic of a Steffensen-type swim tunnel). It was situated horizontally within a larger water tank and the temperature of the recirculating seawater in the tank was controlled at 14 +/- 0.1℃. The rectangular working section of the swim tunnel is 28cm long, 7.5cm wide, 7.5cm deep. Water flow inside was redirected and

laminarized by baffles and a honeycomb at the inlet of the working section and flow speed was calibrated from 0 to 125 +/- 10 cm/s using a Hoentszch TAD flow meter. The solid blocking effect was corrected automatically in AquaResp Swim Software based on the fish length, depth and width (Bell and Terhune 1970). The water was circulated from the system into a sump-bucket where the water was oxygen saturated by an airstone. A separate cooling circulation maintained the temperature in the sump-bucket on 13.8 °C, by pumping (EHEIM pump) water through a cooling coil placed in a cooling tank (6℃), if needed. From the sump-bucket the water was pumped through a UV-filter and back into the respirometer tank.

Measurements of oxygen consumption (MO_2) were taken every second during a 10 minute period which includes a 240s flush, a 60s wait and a 300s measurement, which gave 3 measurement periods for every swimming speed. Oxygen calibration was carried out by using air-saturated seawater as 100% and sodium sulfate as 0%.

A computer monitor of 37 cm L * 32cm H (1280*1024 resolution) was placed parallel to the flow direction next to the working section. Two animations, either of a blank working section or an approximately 8cm long *cymatogaster aggregata* swimming at 1.5 Bl/s was shown on the screen depending on the experimental trial. To minimise disturbance of the fish a 73 cm L* 60cm H board was placed close to the swim tunnel on the opposite side of the monitor to avoid fish seeing external stimuli during the experiments. Above the swim tunnel a GoPro HERO 4, and an LED light was placed, as to record the dorsal view of the fish throughout the trials at 60 frames/s and 1080 resolution.

Protocol

Fish were randomly assigned to one of three treatments, referred to here as “Single”, “Pair” and “False pair”. Single fish were swum in the flume by themselves with a computer monitor alongside one wall of the flume showing an image of the empty flume. Pair trials involved swimming two fish in the flume simultaneously, again with an image of the empty flume on a monitor outside one wall of the flume. False pair trials involved a single fish swimming in the flume by themselves with a computer monitor alongside one wall of the flume showing continuous looped video of a fish swimming in the flume at 1.5 Bl/s.

All fish were measured (standard length, width of the widest point of the body, depth of the deepest point on the body) and weighed prior to a trial. The fish were acclimated in the flume for 5-10 hours at 0.5 Bl/s prior to experimental procedure, to let the effects of handling stress wear off. Fish were said to be acclimated when their MO_2 consumption decreased to a routine metabolic rate that was asymptotic to zero.

The trial consisted of a critical swimming speed (U_{crit}) trial (Brett 1964), with a velocity increment of 1 Bl/s and a time increment of 30 minutes. The speed was increased until the point when the fish were fatigued as evidenced resting on the downstream grill of the flume for a period of 5 seconds during a 30 second period. Respirometry was undertaken during each trial, with 3 measurements of MO_2 at each speed increment. The measurement period for respirometry was 300 seconds, with a 240 seconds flushing period and a 60 seconds

waiting period beforehand. Solid blocking was automatically corrected for within the Autoresp respirometry software using the Bell and Terhune (1970) correction.

At the end of each trial the fish were given 20 - 30 minutes to recover in the flume at 0.5 Bl/s before they were removed to a holding tank.

Kinematic analysis

Gait transition and pectoral fin beat frequency was determined frame-by-frame on the video recordings (GoPro 4) from the individual experiments. The pectoral-caudal gait transition speed (U_{pc}) was defined as the lowest speed at which the tail was used for propulsion for a minimum of 5 seconds per 30 second interval. For pectoral fin beat frequency, the average fin beat count (in beats/s) of three sequences of 10 seconds from every swimming speed from 0.5 Bl/s to post U_{pc} was used for every fish.

Data analysis

MO_2 at each swimming speed was determined for each trial as the mean of the 3 measurements of MO_2. At the highest speed of each trial, MO_2 was taken as either the sole measurement at that speed or the mean of the 2 or 3 measurements at that speed. To derive the standard metabolic rate for each treatment, a power relationship was fitted to MO_2 measurements for each treatment (Videler 1993): $MO_2 = a\,U^b + c$. To assess the statistical significance of any differences in the relationship between MO_2 and swimming speed between treatments, MO_2 was log transformed and a linear regression between log(MO_2) and swimming speed was fitted.

The data of fish fin beat frequency, fish size and weight and oxygen measurements were compared among three different experimental trials by using one-way ANOVA. A post-hoc Tukey's HSD test was used for further analysis when significant differences were discovered. Statistical analyses were undertaken in Statistica and Matlab.

Results

Maximum swimming speed and kinematics

The critical swimming speed, U_{crit} for the single treatment was 5.53 Bl/s (SE ±0.27), 5.27 for the pair treatment (SE ±0.17), and ±5.36 for the false pair treatment (SE 0.31) (Figure 1). The single fish treatment had a slightly greater critical swimming speed than the pair and false pair. Gait transition speed, U_{pc} was close to U_{crit} for all treatments, between (4.40 ± 0.29 single, 4.16 ± 0.14 pair, 4.48 ± 0.33 false pair) (Figure 2). It was highest for the single fish, followed by the false pair and then the pair. These relationships were not statistically significant. Fin beat frequency was similar for single and false pair treatments and for the front fish in a pair at speeds up to 3.5 Bl/s Figure 3). At a swimming speed of 4.5 Bl/s the fin beat frequency was noticeably higher for pair and single fish than for fish swimming in a false pair.

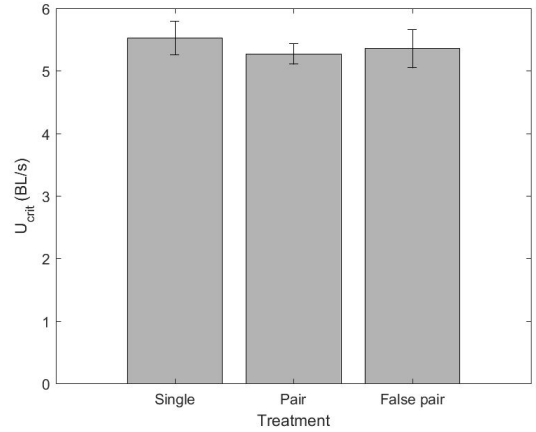

Figure 1 - Critical swimming speed for the single, pair and false pair treatments.

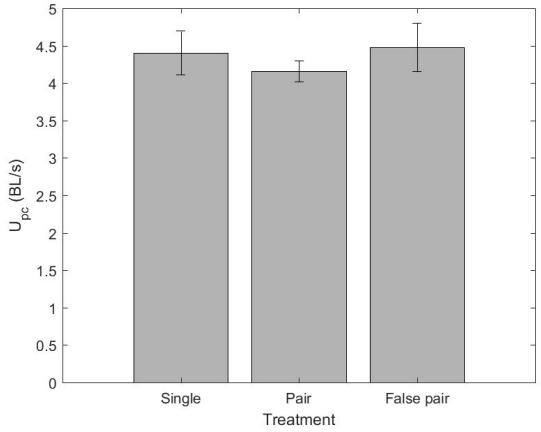

Figure 2 - Gait transition speed (U_{pc}) for the single, pair and false pair treatments;

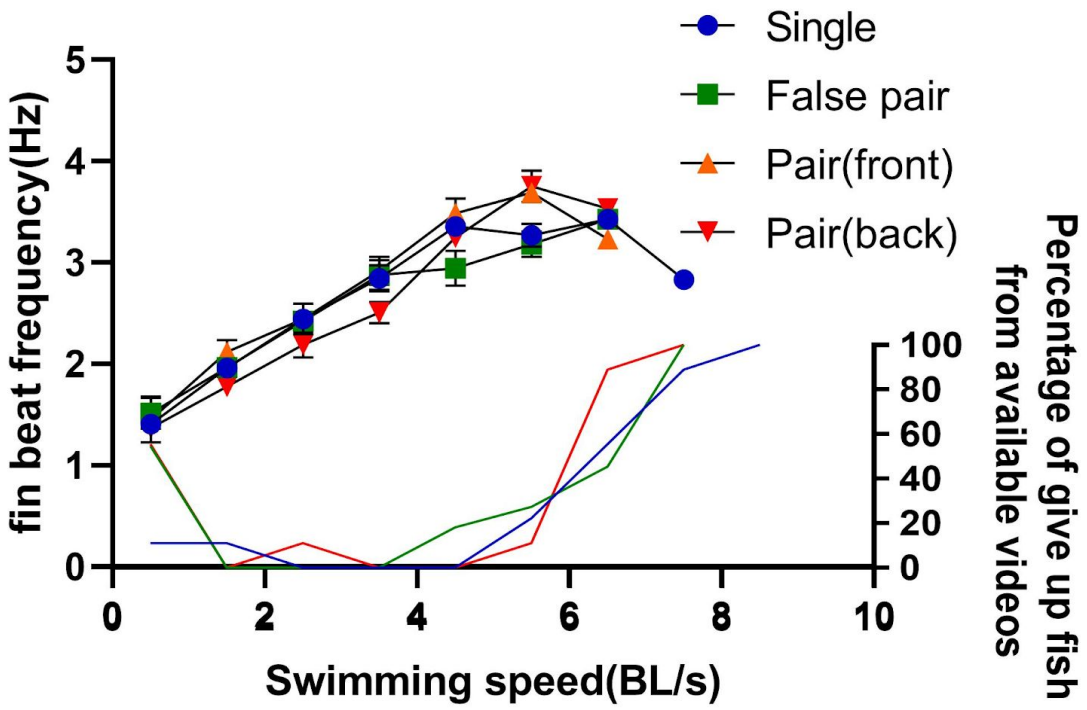

Figure 3 - Fin beat frequency at each speed increment for the single, pair and false pair treatments.

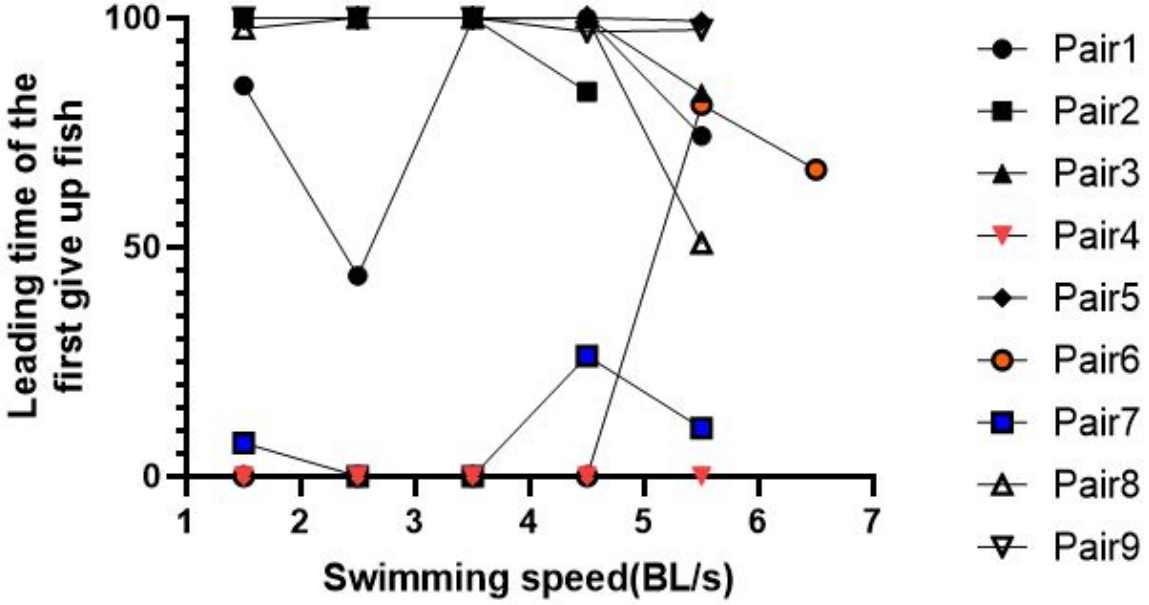

Figure 4 - The leading time of the fish which gave up swimming first in pair treatments. It indicates that in pair treatments 4, 6 and 7, the fish swimming in the back gave up swimming earlier than the fish in front.

Metabolics

The standard metabolic rate of the false pair (181.07 ± 3.16) was highest of all treatments, followed by the single fish (152.82 ± 9.82) and then the pair (142.04 ± 7.41) (Figure 5a). The maximum metabolic rate of the pair was lowest (618.23 ± 35.56), followed by the false pair (682.94 ± 35.61) and then the single fish (717.22 ± 35.61) (Figure 5b). Figure 5 shows the MO_2 measurements at each speed increment and the fitted model of log(MO_2) as a function of swimming speed for each of the treatments. Table 1 presents the statistical information about the fitted relationships. The slope of the Tables 1 to 3 give results for the ANCOVA and linear regression for each treatment relating log (MO_2) to swimming speed. The regressions have a high goodness of fit (R^2 = 0.96 for the single treatment, 0.97 for the pair treatment and 0.92 for the false pair treatment). The ANOVA table (Table 3) shows a test for the significance of the Treatment * Swimming speed interaction, i.e. the difference in slopes of the relationship between log(MO_2) and swimming speed between treatments. With an *F* statistic of 6.57 and a *p* value of 0.0018, the slopes are significantly different.

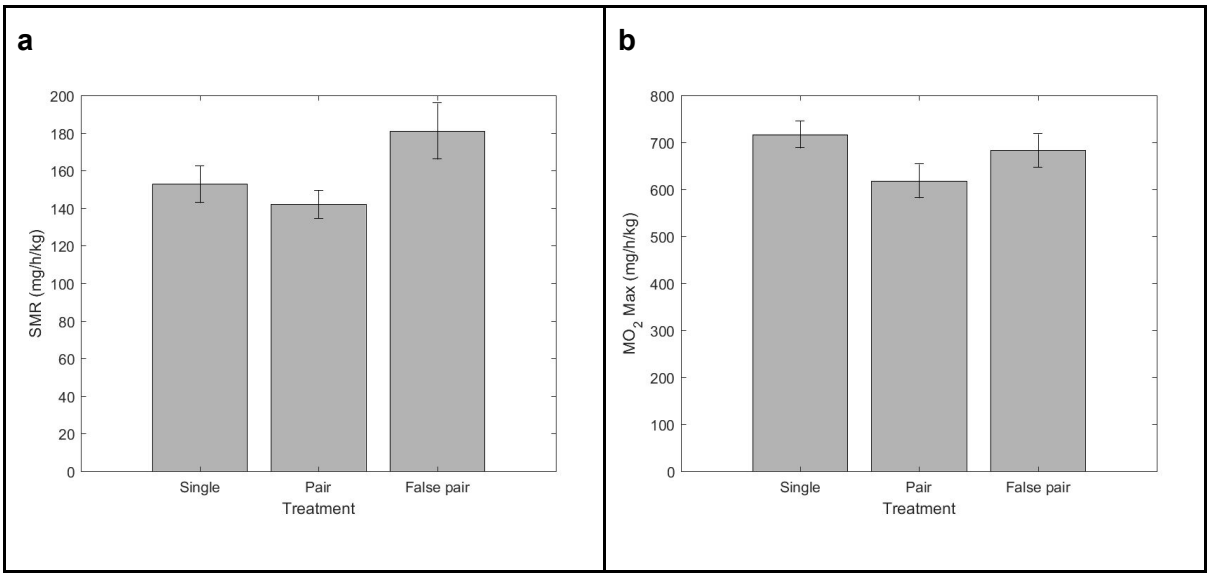

Figure 4 - a) Standard metabolic rate for the single, pair and false pair treatments; b) Maximum metabolic rate for the single, pair and false pair treatments.

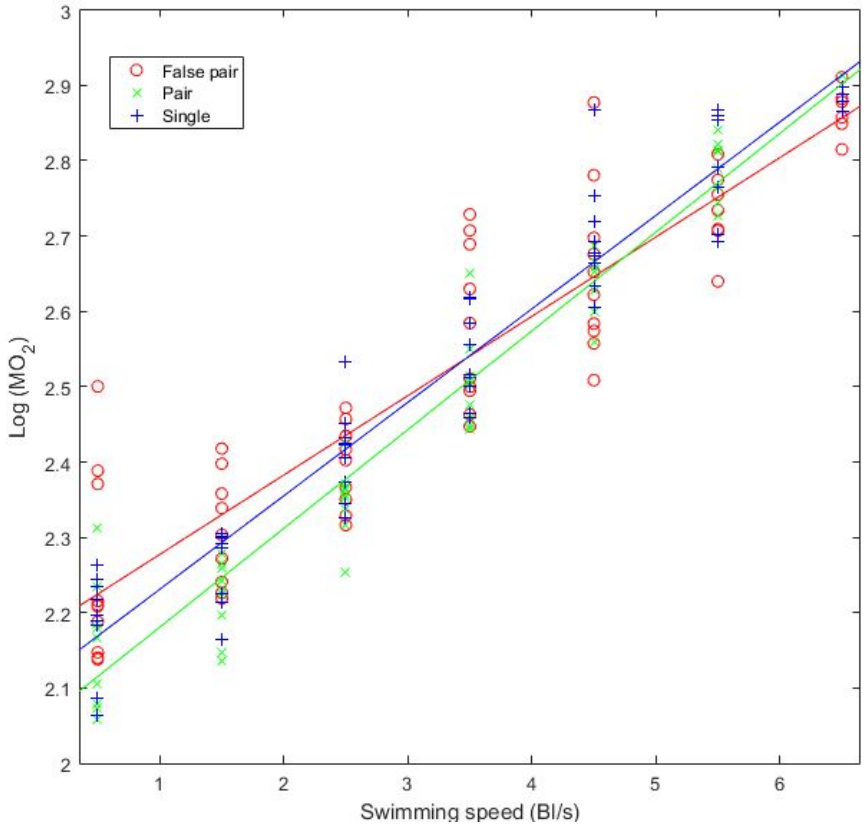

Figure 3 - Measured and modelled oxygen consumption(Log(MO_2)) for the single, pair and false pair treatments, in relation to swimming speed (Bl/s).

Table 1 - ANCOVA coefficient estimates comparing linear regressions for each treatment fitted to log (MO_2) as a function of swimming speed.

Term	**Estimate**	**Std. Err.**	**T**	**Prob >|T|**
Intercept				
Single	2.11	0.03	190.13	0.8704
Pair	2.05	0.03	186.55	0.0003
False pair	2.17	0.03	194.38	0.0001
Slope				
Single	0.12	0.01	39.97	0.3694
Pair	0.13	0.01	41.48	0.0172
False pair	0.11	0.01	35.52	0.0005

Table 2 Goodness of fit for linear regressions for each treatment fitted to log (MO_2) as a function of swimming speed.

Treatment	**R Square**
Single	0.96
Pair	0.97
False pair	0.92

Table 3 ANOVA table for linear regressions for each treatment fitted to log (MO_2) as a function of swimming speed.

Source	**d. f.**	**Sum Sq**	**Mean Sq**	**F**	**Prob > F**
Treatment	2	0.06	0.03	5.48	0.005
Swimming speed	1	8.18	8.18	1518.87	0
Treatment * Swimming speed	2	0.07	0.04	6.57	0.0018
Error	166	0.89	0.01		

Discussion

Comparing results from the false pair treatment with the pair treatment helps to disentangle the hydrodynamic effects of schooling from the behavioural effects, while comparison of these two treatments with the results from the single fish provides a guide as to the overall kinematic and metabolic differences between individual swimming and swimming in a school.

The fish in a false pair tended to start out at 0.5 Bl/s with a higher fin beat frequency than the single fish. From 1.5 to 3.5 Bl/s only the back fish in a pair had a lower fin beat frequency than the front fish or other treatments. However at 4.5 Bl/s and 5.5 Bl/s the false pair had a lower fin beat frequency. This may be because the “fake friend”/GIF swims with 1.5 Bl/s at all speeds, which might manipulate the fish in the swim tunnel to use more energy to keep an eye on the “faster” swimming conspecific (fake friend/GIF) at low speeds and the “fake friend”/GIF calms it down at fast speeds. This tells us that fish use visual stimuli and are aware of their surroundings even when swimming at high speeds, and that there may be a calming effect of swimming with another calm individual when swimming at challengingly high speeds. The observation of a fish swimming with a lower fin beat frequency may influence the neighbouring fish to increase fin beat amplitude and lower it’s fin beat frequency. Mussi et al. (2002) observed that *C. aggregata* had a higher U_{pc} than two other MPF swimming species because of an increased fin beat amplitude rather than frequency.

Comparison of fin beat frequency between front and back fish in a pair showed that the back fish of the pair in a trial had a lower fin beat frequency than the front fish. This demonstrates that there are hydrodynamic advantages in being the rear fish in a swimming pair, and that the rear fish does not use as much energy on swimming. This finding concurs with Johansen et al. (2010) who found that the rear fish in a school of fish swimming using MPF propulsion had c. 15% slower fin beat frequency than front fish, and were estimated to have a 25% reduction in oxygen consumption. Johansen et al. (2010) further determined that schooling would be energetically advantageous in *Embiotoca lateralis*, another MPF swimmer, if more than 78% of individuals in a school occupied trailing positions in the school, which they considered likely given observed schooling of that species. Wiwchar et al. (2017) similarly concluded that schooling provided advantages in swimming performance, finding that U_{crit} was higher in zebrafish tested in a school than those tested alone. However, contrary to these studies, Havs and Oppedal (2019) found that in salmon, schooling did not improve U_{crit}, so the effect may be species specific.

The semi-log linear regression for MO_2 with swimming speed concurred with the fin beat frequency results in pointing to a behavioural advantage of swimming in a pair at high speeds, with the MO_2 of the false pair being the lowest of the three treatments from a swimming speed of c. 2.8 Bl/s up to 3.5 Bl/s. By contrast, the SMR of the false pair treatment was higher than either the single or paired fish. One possible explanation for this is that the active swimming of the “fake fish”/GIF kept the fish in the tunnel more active than it would otherwise have been. Nadler et al. (2016) found that there was a calming effect of shoaling on gregarious fish that depressed their standard metabolic rate. The effect was highly variable between individuals and in the range 5% - 60% of standard metabolic rate. If such a calming effect also occurred in *C. aggregata* and did not vary with activity level then the effect would be of the order 1 - 15% of the maximum metabolic rate, which would likely be within the range of error in the observations. This may explain why the measure of MMR did not show any statistically significant difference between the pair and false pair treatments.

Our study had a number of limitations. In particular, the U_{crit} trials for pairs of fish were terminated when the first fish could no longer keep swimming. This skews downward the measure of U_{crit} for the pair treatment in a way that does not reflect on the effect of schooling on maximum swimming speed. Figure 4 presents the percentage of leading time in relation to which fish gave up first in the pair treatment. In 3 out of 9 trials (~33.3%) it was the trailing fish who gave up first, but in the rest (6 out of 9 ~ 66.6%) it was the leading fish which gave up first. This could indicate that the trailing fish uses less energy than the front fish and therefore there is a hydrodynamic effect in trailing.

In addition, the measurements of oxygen consumption for the pair of fish are necessarily an average of the consumption of the two individuals and the metabolic cost of swimming can not be equally apportioned between the front and rear fish. Figure 5 presents the percentage of swimming fish alongside the fin beat frequency observations. From this graph it can be seen that as U_{crit} was typically between 5 and 5.5 Bl/s, the differences between the fin beat frequency at speeds of 5.5 Bl/s and 6.5 Bl/s may be spurious because few fish swam at those speeds. Fish in all treatments has a tendency to swim near the wall, possibly because the GIF next to the swim tunnel gave the illusion that the tunnel was twice its actual width.

This does mean that fish benefited from slower water speeds in the boundary layer by this wall, but also that they may have been hampered by the inability to fully extend the pectoral fin on that side. This swimming behaviour means that absolute values of U_{crit} and other measures should be interpreted cautiously. However as it was a behaviour observed in nearly all fish and evenly amongst treatments, it does not affect the interpretation of the inter-treatment observations.

While preliminary statistical analysis did not reveal any significant relationships between swimming alone, in a pair, or in a false pair in the gross measures of metabolic and kinematic performance while swimming, the trends in the data suggest that a more thorough investigation is required. In particular, the significantly different slopes in the fitted exponential MO_2 models suggest that at high speeds the metabolic rate in schooling fish may be lower due to the calming effect of swimming in a school. By contrast, at lower speeds the lower pectoral fin beat frequency in the rear fish of a pair compared to the front fish or a single or false pair suggests that hydrodynamic advantages of schooling are dominant over any behavioural effects.

Conclusions

Our results suggest that schooling confers both hydrodynamic and behavioural advantages over swimming alone for a gregarious fish, but that the relative contribution of the two influences depends on the speed of swimming. At low speeds hydrodynamic effects outweigh behavioural effects, but at high speeds when an individual may start to struggle with the effort required to maintain swimming, the presence of another fish who remains calm may have a calming effect on the fish that is struggling.

References

Abrahams, M.V., and P.W. Colgan., 1987, "Fish schools and their hydrodynamic function: a reanalysis." *Environmental Biology of Fishes* 20.1: 79-80.

Bell, W.H., and L.D.B. Terhune, 1970, “Water tunnel design for fisheries research.” *Fisheries Research Board of Canada Technical Report* 195: 1-69.

Drucker, E., and J. Jensen.,1996, “Pectoral Fin Locomotion in the Striped Surfperch. II. Scaling Swimming Kinematics and Performance at a Gait Transition.” *The Journal of Experimental Biology*, vol. 199, no. Pt 10, pp. 2243–52.

Johansen, J. L., Vaknin, R., Steffensen, J.F., Dominici, P., 2010, “Kinematics and Energetic Benefits of Schooling in the Labriform Fish, Striped Surfperch, *Embiotoca Lateralis*.”

Marine Ecology Progress Series, vol. 420, pp. 221–29, doi:10.3354/meps08885.

Klyashtorin, L.V., and R.F. Salkzyanov, 1980, “A Change in Metabolic Rate in Time and the Influence of the Group Effect.” *Journal of Ichthyology*, vol. 20, pp. 132-137.

Lefrançois, C., Ferrari, R.S., Moreira da Silva, J., Dominici, P., 2009, “The effect of progressive hypoxia on spontaneous activity in single and shoaling golden grey mullet *Liza aurata.*” *Journal of Fish Biology*, vol. 75, no. 7, pp. 1615-1625.

Liao, J. C., 2003, “Fish Exploiting Vortices Decrease Muscle Activity.” *Science*, vol. 302, no. 5650, pp. 1566–69, doi:10.1126/science.1088295.

Marras, S., Killen, S., Lindström, J., McKenzie, D., Steffensen, J.F., Dominici, P., 2015, “Fish Swimming in Schools Save Energy regardless of Their Spatial Position.” *Behavioral Ecology and Sociobiology*, vol. 69, pp. 219–26.

Nadler, L.E., Killen, S.S., McClure, E.C., Munday, P.L., McCormick, M.I., 2016, “Shoaling Reduces Metabolic Rate in a Gregarious Coral Reef Fish Species.” *The Journal of Experimental Biology*, vol. 219, no. Pt 18, pp. 2802–05.

Parker, F., 1973, “Reduced Metabolic Rates in Fishes as a Result of Induced Schooling”, *Transactions of the American Fisheries Society,* vol. 102, no. 1, pp. 125-131.

Partridge, B. L., and T. J. Pitcher, 1979, “Evidence against a Hydrodynamic Function for Fish Schools.” *Nature*, vol. 279, no. 5712, May 1979, pp. 418–19.

Ross, R.M., Backman, T.W.H., Limburg, K.E., 1992, “Notes: Group-Size-Mediated Metabolic Rate Reduction in American Shad.” *Transactions of the American Fisheries Society*, vol. 121, no. 3, pp. 385–90.

Walker, J., and M. Westneat, 1997, “Labriform Propulsion in Fishes: Kinematics of Flapping Aquatic Flight in the Bird Wrasse *Gomphosus Varius (Labridae)*.” *The Journal of Experimental Biology*, vol. 200, no. Pt 11, pp. 1549–69.

Webb, P. W., 1984, “Body Form, Locomotion and Foraging in Aquatic Vertebrates.” *American Zoologist*, vol. 24, no. 1, pp. 107–20, doi:10.1093/icb/24.1.107.

Weihs, D., 1973, “Hydromechanics of Fish Schooling.” *Nature*, vol. 241, no. 5387, pp. 290–91, doi:10.1038/241290a0.

Wilson B., Batty R.S., Dill L.M., 2004., “Pacific and Atlantic herring produce burst pulse sounds.” *Proceedings of the Royal Society of London. Series B: Biological Sciences* 271, S95–S97. https://doi.org/10.1098/rsbl.2003.0107

Wiwchar, L.D., Gilbert, M.J.H., Kasurak, A.V., Tierney, K.B., 2017, “Schooling improves critical swimming performance in zebrafish (*Danio rerio*).” *Canadian Journal of Fisheries and Aquatic Science*. 75, 653–661. https://doi.org/10.1139/cjfas-2017-0141