

Eye movements are coordinated with pectoral fin beats during locomotion in a marine teleost fish

Joanna L. Mandecki^{1,*} and Paolo Domenici²

¹Department of Organismal Biology and Anatomy, University of Chicago, 1027 East 57th Street, Chicago, IL 60637, USA

²CNR-IAMC, c/o International Marine Centre, Loc. Sa Mardini, 09072 Torregrande, Oristano, Italy

*Corresponding author: jmandeck@uchicago.edu

ABSTRACT

Animals must simultaneously engage multiple functional systems in order to navigate, feed, and survive in complex environments. Nearly all vertebrates perform rapid gaze-shifting eye movements called saccades, but we know little about the behaviour of saccades during rhythmic locomotion. This study examines how saccades are coordinated with locomotor movements in a pectoral-fin-propelled teleost fish, *Cymatogaster aggregata*, the shiner surfperch. Twelve individuals were filmed swimming in a flow tank at 10 cm s^{-1} , and timing data were analyzed using circular statistics. Results reveal that *Cymatogaster* generates saccades non-uniformly throughout the pectoral fin cycle. Saccades primarily occur during fin abduction, when a large amount of thrust is produced, and rarely occur during the thrust-free refractory phase. Because vision is known to be impaired during saccades, we hypothesize that *Cymatogaster* synchronizes saccades with periods of high acceleration in order to stabilize retinal images during low-acceleration phases, which are nearly saccade-free.

KEYWORDS

locomotion, pectoral fin swimming, coordination, vision, image stabilization, fish

INTRODUCTION

Animals successfully and efficiently execute complex behaviours by synchronizing multiple physiological systems. For instance, parrotfishes coordinate body, fin, jaw, and eye movements during a feeding event to optimize prey capture performance (Rice and Westneat, 2005), and birds and bluegill sunfishes synchronize respiration with locomotor movements to improve energetics (Funk et al., 1997; Tytell and Alexander, 2007). However, these examples describe the coordination of either (1) multiple episodic movements, *i.e.* those that occur occasionally and irregularly, as in the case of feeding parrotfishes, or (2) multiple rhythmic movements, those that occur cyclically and regularly, as in the example of coordination between breathing and locomotion in birds and bluegill sunfishes. We know little about couplings between episodic events and rhythmic locomotion.

Much animal behaviour and locomotion research emphasizes the role of vision and sensory feedback in guiding movement. Nearly all vertebrates perform physiologically-locked reflexive eye movements that serve to compensate for movements of the head or body, thereby stabilizing the retinal image (Masseck and Hoffmann, 2009). This category of eye movements, which includes the vestibulo-ocular reflex and optokinetic response, has been well studied (Carpenter, 1988). Visual saccades, on the other hand, are rapid repositioning eye movements that shift an animal's gaze to another region of space. The eyes move so rapidly that little to no visual information is processed while the eye is in motion (Ebenholtz, 2001). Few studies have focused on the behaviour of visual saccadic movements during steady, rhythmic locomotion, which is surprising given that most vertebrates use vision as a major sensory strategy.

Here we explore the relationship between visual eye saccades and rhythmic locomotion in *Cymatogaster aggregata* Gibbons, the shiner surfperch (Teleostei: Embiotocidae). *Cymatogaster* is well-suited for this study because it beats both pectoral fins concurrently during steady swimming and it maintains a relatively steady position in a current while scanning for prey items. This study examines whether the distribution of saccades is uniform across the three phases of the pectoral fin cycle [abduction, adduction, refractory phase (an idle phase during which the fins are held against the body) (Webb, 1973)] in order to test the hypothesis that saccades may be synchronized preferentially with active locomotor phases that are suboptimal for vision. Fish were filmed swimming in a flow tank at 10 cm s^{-1} using a high-speed camera at 250 Hz. In each trial, frame numbers corresponding to the start and end of each saccade and to the transitions between pectoral fin phases were noted (Fig. 1A). Data were analyzed with circular statistics (Batschelet, 1981)

using the programs CircStat (Berens, 2009), a toolbox for MATLAB (The Mathworks, Inc., Natick, MA, USA), and Oriana (Kovach Computing Services, Anglesey, Wales, UK).

RESULTS AND DISCUSSION

Cymatogaster generated saccades (duration: 31.6 ± 9.0 ms, mean \pm s.d., $N = 144$) non-uniformly across the three phases of the pectoral fin cycle (duration: 474 ± 117 ms, $N = 144$) (Fig. 1B,C, Suppl. Fig. 1). Saccades were generated about once every 2.56 ± 1.12 fin beats ($N = 144$). Each individual's saccade distribution was analyzed using circular statistics. Distributions were significantly different from uniform in 11 of 12 individuals (Rayleigh test: see Suppl. Table 1), with saccades concentrated in fin abduction in all 12 individuals (Suppl. Fig. 1). A second-order analysis performed using the 12 individual mean vector directions confirms that *Cymatogaster* generated saccades primarily during fin abduction (Rayleigh test: $P < 0.001$, $N = 12$) (Suppl. Table 1).

The proportion of saccades occurring in each phase differed from the proportions that would be expected in a uniform distribution [$\chi^2(2, N = 144) = 114.87$, $P < 0.001$]. Overall, 63% percent of saccades occurred during abduction, which lasted 25% of the fin cycle; 20% occurred during adduction, which lasted 29% of the fin cycle; and, most strikingly, only 17% of saccades occurred during the refractory phase, which lasted 46% of the fin cycle (Fig. 1C, with saccade counts shown normalized per phase; see methods). Saccades occurred during pectoral fin abduction significantly more often than would be expected if saccades were uniformly distributed across the fin cycle (exact binomial test: $P < 0.001$, $N = 144$), and saccades occurred significantly less often than expected both during the refractory phase (exact binomial test: $P < 0.001$, $N = 144$) and during adduction (exact binomial test: $P = 0.041$, $N = 144$) (Fig. 1C). In addition, the frequencies of successive pectoral fin beats (*i.e.*, the fin beats prior to, concurrent with, and following each saccade) were not statistically different (mean: 2.44 ± 0.8 Hz, $N = 108$), suggesting that the fins do not alter their frequency to accommodate the generation of a saccade [one-way ANOVA: $F(2,105) = 0.664$, $P = 0.517$].

These results are consistent with a fundamental tenet of visual processing, namely, that stable retinal images are required for high-acuity vision. *Cymatogaster* experiences inconsistent optic flow (distributions of motion vectors on the retina) during swimming because each fin phase generates a different magnitude of propulsive force (Webb, 1973). The phase of fin abduction is not well-suited for image stabilization and visual processing because fin-stroke-related accelerations of the body are relatively high (Webb, 1973). It is

therefore possible that, because vision is impaired during saccadic rapid eye motion (Ebenholtz, 2001), saccades are performed preferentially during abduction, since during this phase visual processing is suboptimal regardless. Body accelerations occur during adduction as well, but the fish during adduction is already moving forward, whereas during abduction the fish transitions from a backward to a forward trajectory, thereby experiencing more “turbulence.” This may explain why eye movements occur more often during abduction than during adduction. Of the three phases, the refractory phase is arguably the phase best suited for visual processing because the fins are not generating any thrust (Webb, 1973). It is possible that saccades are not performed during the refractory phase in order to maximize the time available for optimal visual processing. Furthermore, analyzing retinal images during the refractory phase would allow the fish to initiate a motor command that might require precision fin movements (e.g., a feeding, braking, or turning maneuver) in advance of fin motion.

Image stabilization is the mechanism behind another example of behavioural synchrony found in vertebrates, *i.e.* head-bobbing, a non-locomotor (non-propulsive) behaviour commonly observed in chickens and other birds. Similarly to the locomotor cycle of *Cymatogaster*, a head-bob consists of a thrust phase, when the bird rapidly thrusts its head forward, and a hold phase, when the head is held in a fixed position in space as the body catches up. Eye movements, though generated sporadically, occur predominantly during the thrust phase, when head acceleration is greatest and visual acuity is already limited by a blurry retinal image (Wohlschlager et al., 1993). This allows the bird to maintain gaze and process visual information during the stable hold phase. This example supports the functional hypothesis that *Cymatogaster* restricts its exploratory visual behaviour (*i.e.* the generation of saccades) to a particular phase of the locomotor cycle in order to maximize time for clear (saccade-free) image analysis during another phase.

The coordinated oculomotor-locomotor behaviour seen in *Cymatogaster* is unlike other eye-movement behaviours described in vertebrates. First, it differs from spontaneous scanning eye movements, which are non-visually-evoked eye movements that occur when an animal is motionless (Fritsches and Marshall, 2002; Mensh et al., 2004). These eye movements are seen in many vertebrates (Carpenter, 1988), including fishes such as the goldfish, pipefish, and sandlance (Easter, 1971; Fritsches and Marshall, 2002; Mensh et al., 2004). In *Cymatogaster*, eye movements show a statistical bias in being timed with a distinct phase of the rhythmic locomotor movement. Though parrotfishes stereotypically rotate their eyes laterally just prior to a feeding bite, they do not integrate eye movements with rhythmic

movements but with other episodic fin, jaw, and body movements (Rice and Westneat, 2005). Second, the preferential coordination of the eyes and pectoral fins in *Cymatogaster* differs from the physiologically-locked behaviour of reflexive compensatory eye movements, such as the vestibulo-ocular reflex and the optokinetic response, which are observed in various species of vertebrates (Masseck and Hoffmann, 2009). For instance, the dogfish *Squalus acanthias* coordinates eye movements with axial undulation, but these eye movements are compensatory, driven by the lateral head swing that accompanies each rhythmic undulation (Harris, 1965). The evidence of preferential coordination in *Cymatogaster* suggests that between these two types of behavioural patterns, *i.e.* spontaneous and reflexive eye movements, there exists a spectrum of oculomotor-locomotor linkage strengths.

The coordination between saccades and the pectoral fins described in *Cymatogaster* is distinctive among vertebrates in that it (1) ties episodic eye movements to rhythmic locomotion and (2) demonstrates an intermediate level of coupling between oculomotion and locomotion. Given this evidence of an intermediate level of linkage, one would expect that other species may also exhibit to varying degrees a coordination between the eye and locomotor systems. Whether the coordination in *Cymatogaster* is influenced primarily by visual or vestibular stimuli, or through a feed-forward mechanism that relays efference copies of the fish's locomotor pattern, remains to be tested (Masseck and Hoffmann, 2009). This description of a novel system exhibiting an intermediate level of coupling thus offers new opportunities in which to explore evolutionary, functional, and neural mechanisms of sensory-motor coordination.

MATERIALS AND METHODS

Twelve sub-adult *Cymatogaster aggregata* Gibbons individuals (9.30 ± 0.78 cm, standard length \pm s.d.) were held in flow-through seawater tanks at ambient water temperature ($13.78 \pm 0.90^\circ\text{C}$) and ambient light regime at Friday Harbor Laboratories, University of Washington, in July 2009. Experiments were carried out in a flow tank with workable dimensions of 62 cm in length, 24 cm in width, and 20 cm in water depth. Each individual was introduced to the flow tank and acclimated for 10 min under slowly increasing flow speed and then for 20 min at the experimental flow speed of 10 cm s^{-1} ($0.81\text{--}1.05$ length s^{-1}). At this flow speed, *Cymatogaster* beats the right and left fins synchronously (Webb, 1973) and has been shown to scan for food and feed readily (Mussi et al., 2005). Fish were filmed from a dorsal view using a high-speed camera (Fastec Ranger RA500ME, San Diego, CA, USA) at 250 Hz and 640×480 pixels.

For each individual, 12 sequences of steady swimming were recorded. From each sequence, one horizontal saccade (amplitude > 5 deg) was analyzed. Only trials with minimal or no lateral head or body movement were considered in order to reduce the influence of small lateral shifts that sometimes accompany gaze shifts in vertebrates broadly. In each trial, frame numbers corresponding to the start and end of each saccade and to the transitions between pectoral fin phases were noted. The start of a saccade was defined as the frame preceding the first frame in which a change in eye orientation (the plane of the pupil from dorsal view) was detected, and the end of a saccade was defined as the frame following the last detectable movement (these were short and clear events). The start of adduction was defined as the frame preceding the first frame in which the leading edge of the fin was seen to be moving toward the body, the start of the refractory phase as the first frame in which the leading edge was parallel to the midline of the body, and the start of abduction as the frame preceding the first frame in which the leading edge was seen to pull away from the body. The right eye and fin were used unless obscured for some reason, in which case the left was used. All methods were approved by the Animal Care and Use Committee at the University of Washington (IACUC protocol #3018-09).

Data were analyzed using circular statistics (Batschelet, 1981) and carried out using CircStat (Berens, 2009), a toolbox for MATLAB (The Mathworks, Inc., Natick, MA, USA), and the circular statistics software Oriana (Kovach Computing Services, Anglesey, Wales, UK). One fin cycle, subdivided into its three phases (abduction, adduction, refractory phase), corresponds to one full rotation around a circle (Fig. 1B, Suppl. Fig. 1). The duration of each phase is the mean duration calculated either across individuals (Fig. 1B) or per individual (Suppl. Fig. 1). The timing of the start of each saccade within the phase in which it occurred was scaled relative to the mean phase duration of that individual. Circular plots were generated using a custom script written in MATLAB.

Saccade distributions across the pectoral fin cycle of each individual are described by a mean vector with length R , the metric used in this study to indicate strength of coordination. The length of vector R is a measure of concentration that ranges from 0 to 1; a larger value indicates a higher concentration around the direction in which the mean vector points, whereas a value of 0 indicates a perfectly uniform distribution (Batschelet, 1981). For each individual, statistical significance against a uniform distribution was determined using the Rayleigh test for non-uniformity at a critical P -value of 0.05. A second-order analysis to calculate a grand mean vector across individuals was performed using individual fish means.

A Chi-square test was used to determine whether the proportion of saccades occurring

within each phase was in line with the proportion expected in a uniform distribution based on the relative duration of each phase. An exact binomial test with sequential Bonferroni correction (Rice, 1989) was then used to determine whether any individual phases differ significantly from expected. To visualize this, a normalized value of saccades, S_N , was calculated for each phase by dividing the proportion of saccades in that phase (number of saccades in phase / total number of saccades) by the proportion of fin cycle time of that phase (mean duration of phase / mean fin cycle duration) (Fig. 1C). Using this normalized procedure, a value of $S_N = 1$ would be expected for each phase if saccades were distributed uniformly across all phases (Fig. 1C).

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COMPETING INTERESTS

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

J.L.M. and P.D. conceived and designed the study. J.L.M. collected and analyzed the data. J.L.M. drafted the manuscript and P.D. contributed to revisions.

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FIGURE

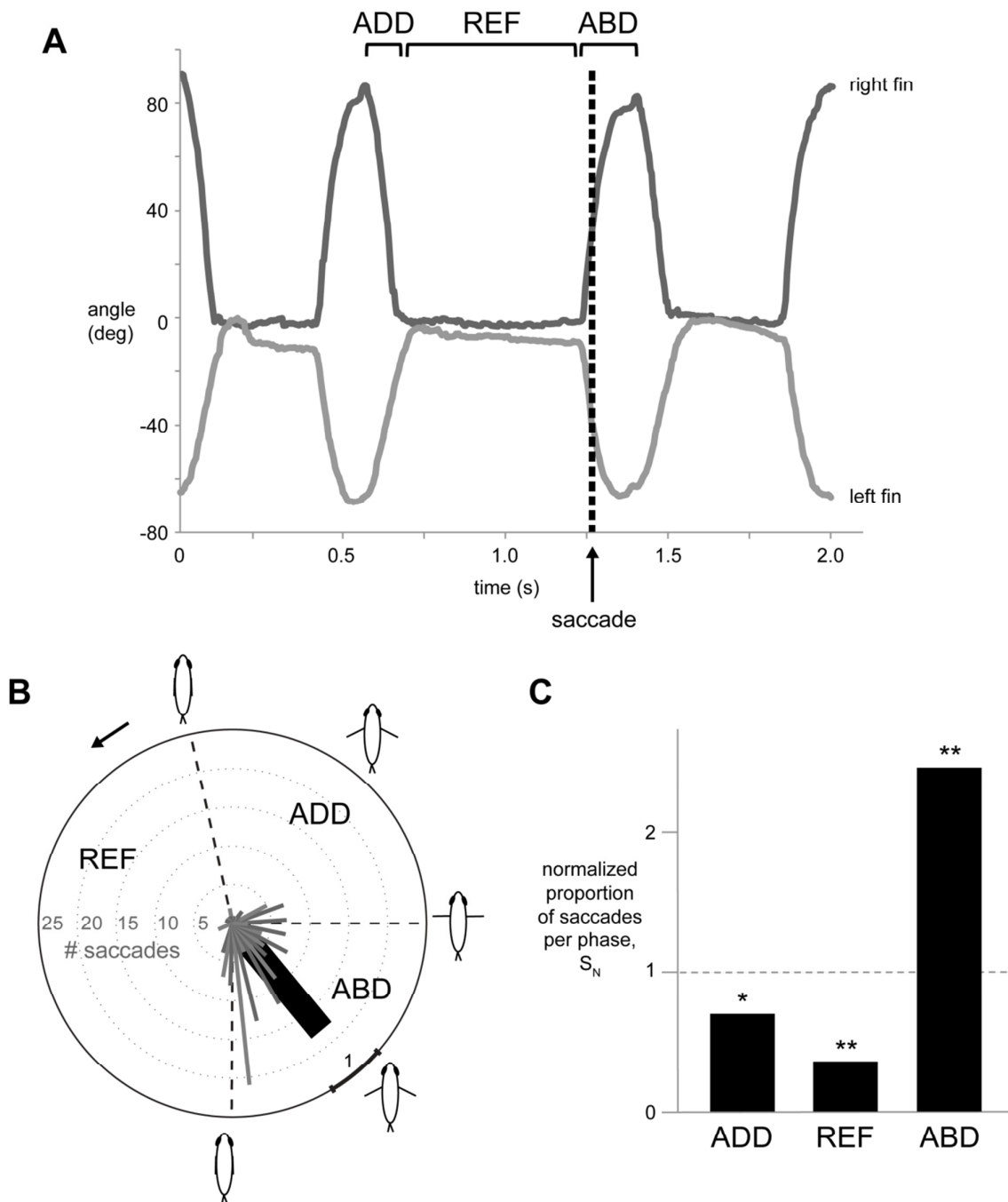


Fig. 1. Saccadic eye movements are coordinated with pectoral fin abduction. (A) A saccade occurs during fin abduction in this representative sequence of three fin beats. The fin traces are for illustrative purposes only and show the angle created between the pectoral fin's leading edge and the midline of the body over time. In the study, fin transition times were

determined visually from the videos (see methods). ABD, abduction; ADD, adduction; REF, refractory phase. (B) A summary circular histogram for all saccade data (grey bars, shown in alternating shades of grey for clarity; $N = 144$) shows saccades occurring primarily during fin abduction. The mean vector R (black bar) points in the direction around which the saccades are concentrated; the length of R represents the degree of concentration on a scale of 0 to 1. The bold arc along the plot's circumference is the 95% confidence interval around the mean-vector direction. Time begins arbitrarily at fin adduction at (1,0) and moves counterclockwise, as shown by the small arrow. Fish diagrams illustrate approximate fin positions. Data are divided into 45 bins. (C) The distribution of saccades deviates from what would be expected in a uniform distribution. During fin abduction saccades occur more often than would be expected, whereas during adduction and the refractory phase they occur less often than expected. For each phase of the fin cycle, the number of saccades is shown normalized to the mean duration of the phase; a value of $S_N = 1$ would be expected if the observed number of saccades were equal to the number predicted in a uniform distribution. *, $P < 0.05$; **, $P < 0.001$ (exact binomial test).