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# Testing the Optomotor Response in Sepia bandensis

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# Testing the Optomotor Response in *Sepia bandensis*

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Biology.

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## ABSTRACT

Cephalopods (octopus, squid, and cuttlefish) have commonly been used as models to test visual function and camouflage due to their similarity in eye morphology with humans and because of their readily observable changes in body color in response to visual stimuli. Most studies have used a single species, *Sepia officinalis*, to make broad conclusions about camouflage and vision. However, these generalizations may not be applicable to all species. Here, I have examined visual function of the dwarf cuttlefish (*Sepia bandensis*), which differs from *S. officinalis* in habitat, geographic range, and size. Using the optomotor response, I quantified the minimum separable angle (MSA) of resolution, a behavioral measure of visual acuity, by recording cuttlefish movement in response to rotating black and white stripes of decreasing stripe width. The threshold of visual acuity for these experiments was a stripe width of 5mm and a MSA of 3.76°. These results indicate that *S. bandensis* has poorer visual acuity than *S. officinalis* (MSA 0.57°), and therefore, may be less able to resolve fine details in the environment. The ability to perceive these fine details enables animals to navigate, forage, and communicate with conspecifics. Future work should examine the behavioral ecology of *S. bandensis* to understand the biological and physical environmental context in which visual cues are used by this species.

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## INTRODUCTION

Visual systems allow organisms to perceive their environment by acquiring visible light from their surroundings. When light strikes the lens of the eye, it is refracted and focused on the retina as an image of the organism's field of view (Dusenbery, 1992). This image is turned into electrical impulses via rods and cones, and sent on to the brain for further processing (Purves et al., 2012). Visual perception aids in locomotion by allowing an organism to recognize obstacles and pathways, and also aids in feeding by allowing an organism to identify and seek out preferred food sources (Dusenbery, 1992). Visual systems vary between species, and the visual abilities of a species are limited by the physical eye structure and physiology with respect to color vision, temporal resolution/ retinal integration speed, and visual acuity/spatial resolution (Dusenbery, 1992).

Temporal resolution indicates an organism's ability to track moving objects; this may be especially important for active predators that require fast eyes to hunt quickly moving prey (Bedore, 2013). Color vision is the ability to distinguish objects by the wavelength of light they reflect and can aid in communication, foraging, and predator

avoidance for animals in spectrally rich habitats (Bedore, 2013). Visual acuity refers to

clarity of the visual image (Tansley, 1965); higher visual acuity means that an organism

can perceive smaller details in its surroundings. Organisms, such as cuttlefish, that rely

heavily on visual cues from their environment to enable accurate camouflage may benefit

from high visual acuity. Vision has also been shown to play a significant role in foraging

cuttlefish (Messenger, 1968).

Optomotor and optokinetic responses are behavioral indicators that can be used to

quantify the limits of the visual system. The optomotor response is a reflexive turning

motion of the head and body that occurs in response to moving stimuli, which functions

to stabilize an image on the retina (Groeger et al., 2005; Talbot and Marshall, 2010;

Caves et al., 2016). A related response, the optokinetic response, typically occurs when

the organism is stationary. During an optokinetic response, the eyes (but not the body)

track the moving stimulus, then move quickly back to their initial position when the

stimulus leaves their field of vision (Purves et al., 2012). Optomotor and optokinetic

response experiments are advantageous because they do not require sacrificing study

animals, as visual physiology studies often do.

Optomotor and optokinetic responses use rotating stripes of alternating colors

and/or brightness as stimuli; movement of the animal in the direction of the stripe rotation

is considered a positive optomotor response, whereas saccadic eye movements in the

direction of the stripe rotation is considered a positive optokinetic response (Caves et al.

2016). Varying black and white stripe width allows for quantification of visual acuity.

There is an inverse relationship between stripe width and visual acuity: the thinner the

stripe width that organisms can distinguish, the greater their visual acuity. This inverse

relationship between stripe width and visual acuity can also be expressed as the minimum separable angle (MSA). MSA is the critical angular spacing of two stimuli when they are just resolved (McIlwain, 1996). MSA is related to photoreceptor spacing and the focal power of the eye (Groeger et al., 2005). Optomotor and optokinetic responses have previously identified polarization sensitivity and ontogenetic change in visual acuity in some cephalopod species (Talbot and Marshall, 2010; Cartron et al., 2013; Groeger et al., 2005).

Cuttlefish are a type of coleoid cephalopod with a high brain-to-body ratio. Pigmented chromatophores in their skin allow them to quickly and easily change their coloration, and they are well known for their camouflage abilities. Because of these abilities, researchers have used cuttlefish as models to learning about sensory perception and camouflage cues. This information can be applied to other species that use visual cues from the environment to coordinate behaviors associated with camouflage, communication, and navigation and orientation.

The species of interest for this work is the dwarf cuttlefish, *Sepia bandensis.* *Sepia bandensis* rarely appears in scientific literature, and no data have been published on their visual capabilities. Much of the current knowledge of cuttlefish vision has been determined from studies of *Sepia officinalis*, the common cuttlefish. These data have been broadly applied to all cephalopod species, but further testing of other cuttlefish species is necessary to determine if visual capabilities are comparable between species. *Sepia officinalis* and *S. bandensis* occupy different geographic ranges, habitats, and grow to different sizes as adults (Barratt and Allcock, 2012). Because of the differences in their size and habitats, it is possible that there are differences in visual capabilities between the

species that have not been previously documented. For example, visual acuity is affected by body size across a range of species including vertebrates and cephalopods; typically, as body size increases, so does visual acuity (Carvalho et al., 2002) (Groeger et al., 2005) (Packard, 1969) (Pankhurst, 1994). Light intensity also affects visual acuity (Hemmi and Mark, 1998; Schmid and Wilsoet, 1998); as light intensity increases, MSA decreases, indicating that acuity increases with light intensity (Groeger et al., 2005). The differences in light habitat for *S. bandensis* and *S. officinalis* may also lead to differences in their visual acuity. Despite their smaller body size, I hypothesize that *S. bandensis* will have a higher visual acuity than *S. officinalis* due to the increased light availability in their natural habitat.

## METHODS

Cuttlefish (n=3) were maintained in individual tanks at a temperature of 23°C, on a 12:12 light: dark cycle, and fed to satiation daily. All experiments were conducted in an optomotor rig (Figure 1) with an inner circular tank for holding individuals (20.3 cm diameter, 10.2 cm tall) and an outer drum (30.5 cm diameter) which rotated to provide the individuals with moving stimuli. The moving stimuli consisted of black and white striped paper with varying stripe widths. Each striped stimulus was printed on printer paper using a Ricoh MP C4502 color printer, laminated, and taped together in order to form a circular paper drum, which was then attached to the clear acrylic outer drum. Attached to the underside of the outer drum was a Nema-34 stepper motor which was computer-controlled using modified Phidgets software to allow for manipulation of

rotational direction and speed of stimuli. All experimental trials in the optomotor rig were video recorded with a Canon G12 camera positioned over the inner optomotor tank so that all cuttlefish motion (optomotor and optokinetic responses) could be quantified. Lamps with 9 watt LED bulbs were used to provide even and consistent lighting in the experimental tank.

*Data Collection*

To test visual acuity, cuttlefish were tested with stripe widths ranging from 1.0-40.0 mm. Stripes increased in 1.0 mm increments for stripes 1.0- 5.0mm wide (excluding 3.0 mm), and in 5.0 mm widths for stripes 5.0- 40.0 mm wide. A solid-colored black drum and solid-colored white drum were included as controls. Choice of stripe width was randomly selected for each trial, and stripes were rotated clockwise and counterclockwise for each trial, with the order of the directions occurring randomly.

For each experimental session, an individual cuttlefish was transferred to the holding tank within the optomotor rig, and allowed a 20-minute adjustment period in the experimental arena. After the adjustment period, the camera was turned on and recorded 30 seconds of activity before stripe movement occurred, after which, the stepper motor was turned on, and the outer drum rotated for 2 minutes in a randomly chosen direction. The motor was then turned off for 1 minute so that no stripe movement occurred. The motor was turned on for an additional 2 minutes in which the drum was rotated in the opposite direction. Finally, the motor was then turned off to end stripe movement and an additional 30 seconds of post-stripe movement were recorded after the motor was turned

off; this signified the end of the trial. If subsequent trials occurred directly after, the adjustment time at the beginning of the trial was shortened to 5 minutes. The striped drum was changed during the adjustment period to present the cuttlefish with new stimuli in the next trial. Speed was maintained at 5 rpm for the entirety of the experiment. All cuttlefish were returned to their tanks at the conclusion of a trial, and were returned to their individual tanks within one hour of their initial removal.

*Video Analysis*

Out of the 240 seconds of total stripe movement recorded per trial, 180 seconds (90 seconds clockwise and 90 seconds counterclockwise) were used for analysis. Cuttlefish movement in the same direction as stripe movement was considered positive movement. Cuttlefish movement in the opposite direction was considered negative movement. For each trial, the amount of time the cuttlefish spent moving in the positive and negative directions was recorded, and the total amount of time spent moving in the positive direction was calculated. If the total amount of time spent moving in the positive direction was greater than 5 seconds, the cuttlefish were considered capable of discerning the stripe width used in the trial. Also, trials in which the cuttlefish made one or more half rotations around the drum in the positive direction were considered further evidence for visual acuity (Caves et al., 2016). The cycles per degree and minimum separable angle (MSA) of the smallest stripe width that elicited a positive response was calculated for each individual. Cycles per degree was calculated using a protractor to determine the fraction of a cycle (one black-white stripe pair) that occurred within an angle of 1° (Figure 2). MSA was calculated using the formula MSA = 2 arc tan (0.5W/RD), where W

represents stripe width (in mm) and RD (mm) represents reactive distance, the distance

between the individual and the stimulus.
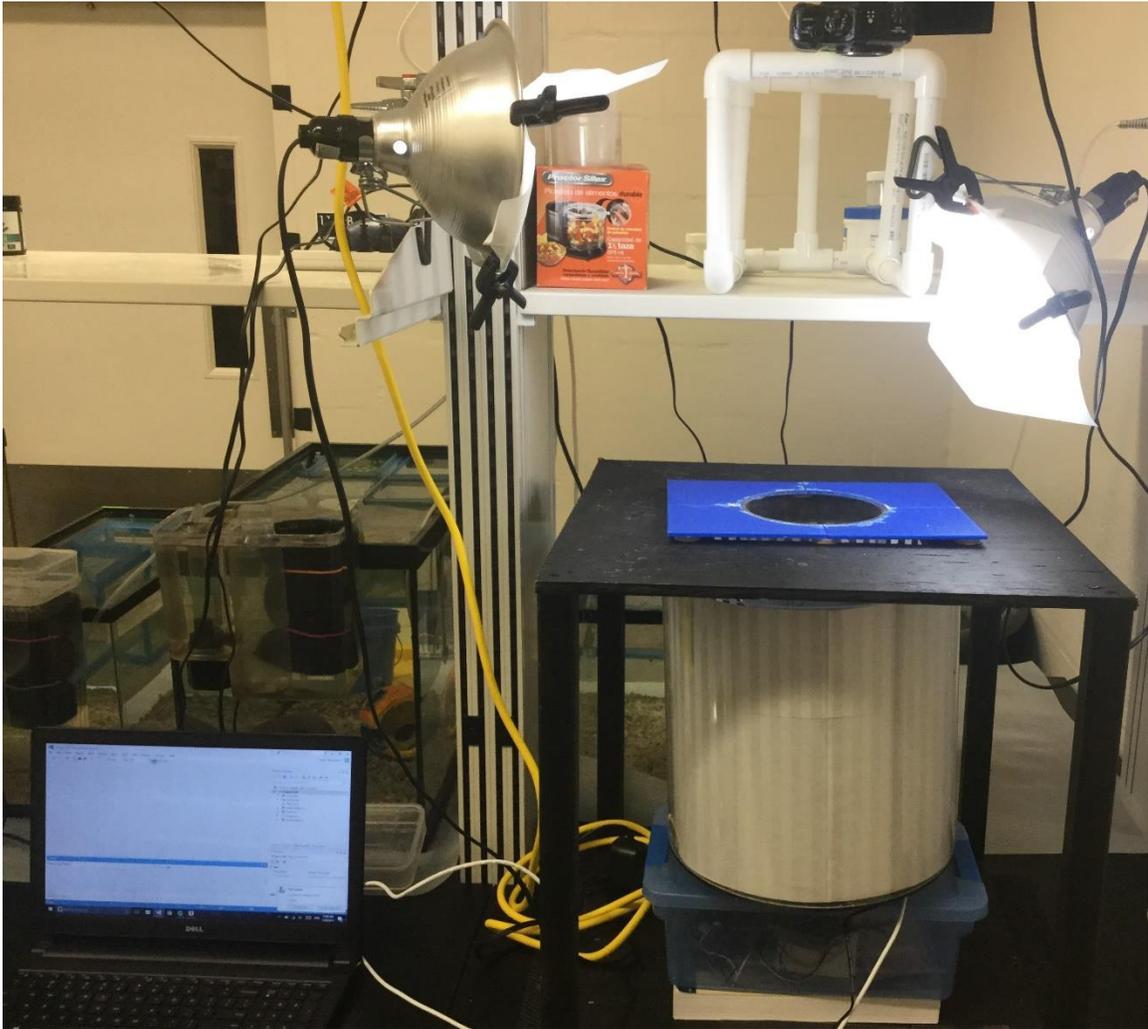


Figure 1. Optomotor set-up used for experiment. A camera was suspended over the
experimental tank (set into table). The outer drum with striped stimuli was mounted on
the stepper motor (enclosed in box for protection from saltwater) by a 3D printed stage.
The stepper motor was connected to a laptop via USB, which controlled the stepper
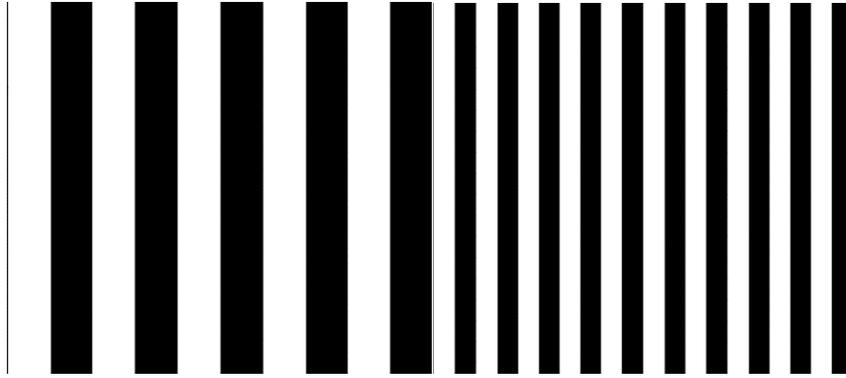motor via modified Phidgets software.

Figure 2. Example of striped stimuli used in trials (not to scale). One pair of black and white stripes is equivalent to one cycle. Decreasing stripe width corresponds to a lower MSA and higher visual acuity.

## RESULTS

On average, cuttlefish demonstrated positive optomotor responses (swimming in the same direction as drum rotation) for >80s of each 90s trial for stripe widths of 5.0 and 10.0mm (Figure 3). In 39.4% of trials, individuals made at least one-half positive rotation in the direction of stimulus rotation. 92.3% of trials where movement in the positive direction was greater than 5 seconds also included one or more half rotation in the positive direction. Combined, these results indicate that *S. bandensis* can differentiate between black and white stripes 5.0 mm or larger.

When the stripe width was less than 5.0 mm, most individuals spent less than 5.0 seconds moving in the positive direction, with the exception of cuttlefish 2, which spent 13 seconds moving in the positive direction when exposed to 4.0 mm stripes. Cuttlefish demonstrated less than 5.0s positive movement in control trials (Figure 2). The average

cycles per degree that elicited a positive response was 0.29 and the minimum separable angle was 3.76° (Table 2; Figure 4).

Table 1. Body size of individual *S. bandensis* used in trials; mantle lengths were determined from still images of video from their first (initial) and last (final) trials. Cuttlefish 3 died during the course of the experiment, before a change in size could occur.

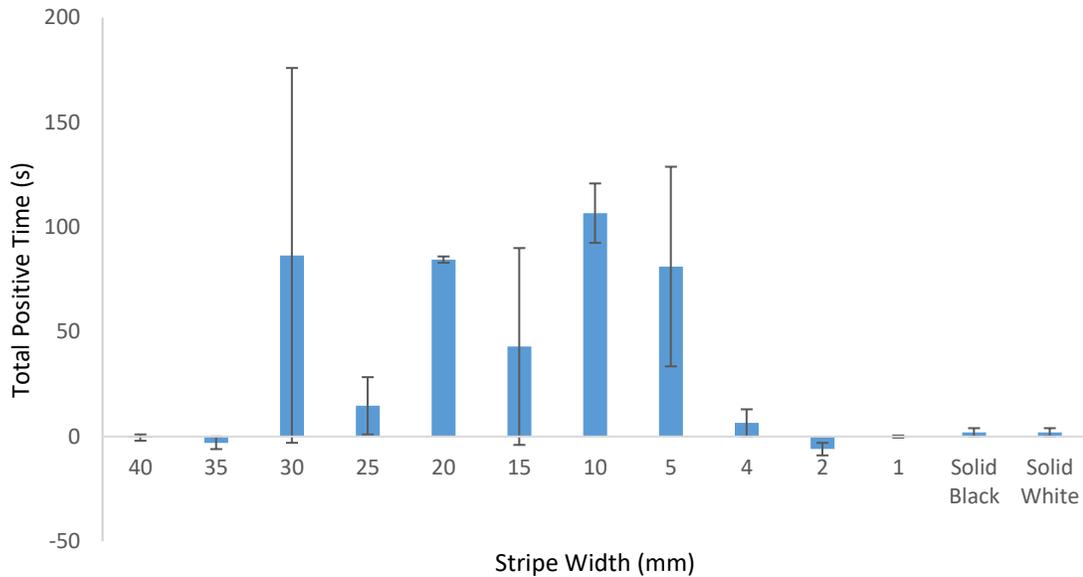| Cuttlefish # | Initial Mantle Length (mm) | Final Mantle Length (mm) |
|---|---|---|
| 2 | 44.7 | 63 |
| 3 | 38.6 | -- |
| 4 | 48.6 | 89.3 |



Figure 3. Total time spent moving in the positive direction (the same direction as stripe rotation). Below stripe widths of 5 mm, individual *S. bandensis* movement did not meet either criterion for positive movement, indicating a potential lack of ability to discern stimuli below this threshold. (n=3, data are means ± SE).
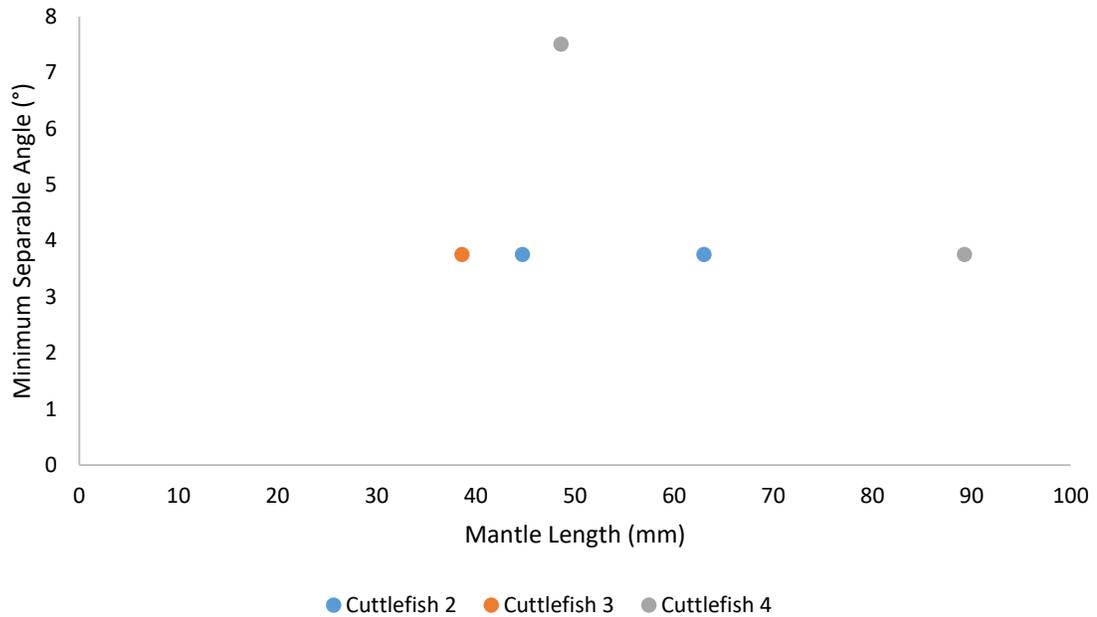
Figure 4. Minimum Separable Angle (MSA) compared to mantle length(s). Individuals in this study did not fit the expected trend of MSA decreasing as mantle size increased. (n = 3)

## CONCLUSIONS

Table 2. Comparison of smallest experimental MSA values in degrees for related species *S. bandensis* and *S. officinalis* (Groeger et al., 2005).

| Species | Minimum Separable Angle (°) |
|---|---|
| *Sepia bandensis* | 3.76 |
| *Sepia officinalis* | 0.57 |

The cuttlefish used in this study (*Sepia bandensis*) demonstrated a larger

experimental MSA than *S. officinalis,* indicating that visual acuity does differ by species.

An inverse relationship has been demonstrated between MSA and body size in

cephalopods, with MSA decreasing as body size increases (Groeger et al., 2005)

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(Packard, 1969). In this study, a 5.0 mm threshold/ 3.76° MSA was demonstrated for

individuals ranging from 40- 90 mm, which is not indicative of an increase in visual

acuity with increased body size (Figure 4). Individuals in this study were tested over the

course of several months (from November 2016 to February 2017), and grew in size

during that time (Table 1). Because of their repeated exposure to stimuli as their body

size increased, acclimation to experiments may account for the lack of change in MSA

with increasing mantle length. Behavioral changes such as increased nystagmic responses

were noted in individuals as they increased in size, which may indicate acclimation.

These may also be due to acclimation or maturation; future ontogenetic studies may

provide insight into how visual acuity changes with respect to body size in this species, as

well as behavioral changes to optomotor stimuli.

*Sepia officinalis* and *S. bandensis* occupy different geographic regions and niches.

*Sepia officinalis* inhabits sandy or muddy substrates of the Mediterranean Sea and

eastern/northeastern Atlantic Ocean, and are a migratory species (Barratt and Allcock,

2012). As juveniles, *S. officinalis* occupy shallower inshore waters; adults occupy deeper

waters (approximately 100 m), but return to shallower water in the spring and summer to

mate (Barratt and Allcock, 2012). Adults can attain a mantle length of 300- 490 mm

(Barratt and Allcock, 2012). *Sepia bandensis* inhabit coral reefs or sandy habitats in the

shallow waters of the Indian Ocean and western central Pacific Ocean (Barratt and

Allcock, 2012). No information on migration patterns or maximum mantle length has

been reported for this species, but individuals maintained in the laboratory attained a

mantle length of approximately 60- 90 mm as adults (Table 1). If adults from each

species were compared directly, there would be a significant difference in body size,

which would indicate that the larger species (*S. officinalis*) should have a better visual acuity than the smaller *S. bandensis*. When values obtained from juvenile *S. officinalis* were compared to those of similarly sized *S. bandensis*, *S. officinalis* still demonstrated higher visual acuity (Table 2). The importance of visual acuity in camouflage and foraging for juvenile *S. officinalis* (Messenger, 1968) may require high visual acuity in order to develop proficient hunting and camouflage techniques before migrating to deeper waters with less light availability.

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# APPENDIX


Appendix A. Time spent moving in same direction as stripe rotation (+) and opposite from stripe rotation (-) for each *S. bandensis* individual for each stripe width. (n=3) Trials in which individuals make at least + ½ rotation of tank are denoted Y in the final column.

| Cuttlefish # | Stripe Width (mm) | (+) Time (s) | (-) Time (s) | Total (+) Time (s) | (+) 1/2 Rotation |
|---|---|---|---|---|---|
| 2 | 40 | 26 | 28 | -2 | Y |
|  | 35 | 20 | 26 | -6 |  |
|  | 30 | 178 | 2 | 176 | Y |
|  | 25 | 16 | 14 | 2 |  |
|  | 20 | 86 | 0 | 86 | Y |
|  | 15 | 2 | 6 | -4 |  |
|  | 10 | 90 | 9 | 81 | Y |
|  | 5 | 90 | 0 | 90 | Y |
|  | 5* | 69 | 52 | 17 | Y |
|  | 4 | 39 | 26 | 13 |  |
|  | 2 | 17 | 26 | -9 |  |
|  | 1 | 30 | 26 | 4 |  |
|  | 1* | 0 | 3 | -3 |  |
|  | SB | 0 | 0 | 0 |  |
|  | SW | 13 | 9 | 4 |  |
| 3 | 40 |  |  |  |  |
|  | 35 |  |  |  |  |
|  | 30 |  |  |  |  |
|  | 25 | 44 | 2 | 42 | Y |
|  | 20 |  |  |  |  |
|  | 15 |  |  |  |  |

| | | | | | |
|---|---|---|---|---|---|
| | 10 | 130 | 0 | 130 | Y |
| | 5 | 179 | 5 | 174 | Y |
| | 4 | | | | |
| | 2 | | | | |
| | 1 | | | | |
| | SB | | | | |
| | SW | | | | |
| 4 | 40 | 4 | 3 | 1 | |
| | 35 | 0 | 0 | 0 | |
| | 30 | 21 | 24 | -3 | |
| | 25 | 0 | 0 | 0 | |
| | 20 | 89 | 3 | 83 | Y |
| | 15 | 90 | 0 | 90 | Y |
| | 10 | 126 | 17 | 109 | Y |
| | 5 | 15 | 27 | -12 | |
| | 5* | 59 | 15 | 44 | Y |
| | 4 | 0 | 0 | 0 | |
| | 2 | 0 | 3 | -3 | |
| | 1 | 0 | 0 | 0 | |
| | 1* | 3 | 4 | -1 | |
| | SB | 9 | 5 | 4 | |
| | SW | 24 | 24 | 0 | |