Effect of time of day on escape response in Pacific staghorn sculpin, *Leptocottus armatus*

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ABSTRACT

The present study aims to test both the daily locomotor activity of Staghorn sculpins (*Leptocottus armatus*) and the effect of the time of day on the escape response in this species. The daily locomotor activity of 12 individual fish was evaluated in 2 sets coinciding with two opposite tidal stage in nature (low and high tides). Fish displayed mainly nocturnal daily activity (82.5 % of daily activity occurring at night) during a 24 h cycle under a simulated natural photoperiod of 15 h Light: 9 h Darkness (switch on at 05:30 h and switch off at 21:30 h) independently of the tidal phase in nature. Moreover, the escape response to a mechanical stimulus was investigated at 4 different times of day (at 01:30, 07:30, 13:30 and 19:30 h). Sculpin escape response depended on the time of day since lower responsiveness (20.6% of fish showed escape responses) and longer travelled distance (26.40 cm) occurred at night (01:30 h) than during the daytime (58.8% of mean responsiveness and 16.76 cm as mean travelled distance). In contrast, no statistical differences between times of day were found in some escape response parameters, such as directionality (fish always went away), latency, turning angle and turning rate. These results suggested the elusiveness of sculpin is more reduced during their activity phase (at night), but if they then respond to the stimulus, their fast-start performance is greater in terms of travelled distance.

INTRODUCTION

Escape responses are sudden accelerations in animals provoked by a threatening stimulus, which could be mechanical, acoustic (Eaton & Hackett, 1984; Domenici & Batty, 1997) and visual (Dill, 1974; Batty, 1989; Harper & Blake, 1991). In teleost fish, these escape responses are controlled by Mauthner cells (Eaton & Hackett, 1984), which lead to muscular contraction in the fish body side opposite to the stimulus, showing a 'C-shape' (stage 1) (Domenici & Blake, 1997). After the stage 1 a contra-lateral contraction usually occurs by a return 'flip' of the tail (stage 2). Finally, the stage 3 takes place, being the most variable one (Weihs, 1973). Moreover, 'S-starts' have been observed as escape response, mediated by simultaneous contractions of both side of the axial musculature, showing an 'S-shape' (Hale, 2002). Several factors influence the escape response in fish. Among the intrinsic factors, it is noteworthy that escape response is species-dependent, being determinate by neural drive, muscle mass, body flexibility and shape (Domenici, 2010), although escape responses also vary with the fish size (Wardle, 1975; Webb, 1976; Domenici & Blake, 1993a; Domenici, 2001) and ontogenetic stage (Wakeling et al., 1999; Hale, 1999; Fuiman et al., 1999). Abiotic factors such as stimulus intensity and orientation (Eaton & Hackett, 1984; Domenici & Blake, 1993), temperature (Webb, 1978; Beddow et al., 1995; Webb and Zhang, 1994) hypoxia (Lefrançois et al., 2005) and turbidity (Domenici et al., 2007a) have been also shown to affect different parameters of the escape response in several fish species. Specifically, the escape response in Staghorn sculpin, *Leptocottus armatus*, has been already evaluated with respect to the size influence (Paglianti and Domenici, 2006) and the obstacles presence (Serena et al., 2009). However, the timing influence along the day on the escape performances has not been investigated in Staghorn sculpin. The only evidence of the daily variations in fish escape response has been reported by Li and Dowling (1998), who determined the threshold of zebrafish visual sensitivity along the day by means of responsiveness of escape response.

Daily variations of different biological parameters have been reported in fish depending on the time of day (Lopez-Olmeda and Sanchez-Vazquez, 2010). It is due to almost all organisms have developed molecular clocks to anticipate recurrent changes in their habitat, in order to optimize their survival (Madrid et al., 2001). Molecular clock endogenously controls biological rhythms, since rhythmically expresses clock genes to lead other overt rhythms, including behavioral and physiological rhythms. These biological rhythms are synchronized by cyclic environmental signals, amongst which light/darkness cycles have been considered as the most important one. Therefore, fish often adjust their activity to the photophase or scotophase, being classified as diurnal (the greatest activity occurs during the photophase), nocturnal (the greatest activity occurs during the darkphase) or crepuscular (activity linked to dawn and dusk) (Madrid et al., 2001). Up to now, daily activity in sculpin has not been reported.

The present study aims to observe the daily locomotor activity of the Staghorn sculpin *Leptocottus armatus* and investigate the effect of the time of day on their escape response to a mechanic stimulus. To this aim, non-locomotor (responsiveness, escape latency and travelled distance) and locomotor (directionality, turning angle, turning rate) parameters were evaluated.

MATERIALS AND METHODS

Fish collection and maintenance

A total of 117 Staghorn sculpins (*Leptocottus armatus*, Girard) of 50.6 ± 1.4 g (mean ± Standard Error of Mean, SEM) body weight and 16.3 ± 0.2 cm body length were collected on August 2013, using beach seining off Jackson Beach, San Juan Island, Washington, U.S.A. (48° 30’ N; 123° 06’ W). Upon arrival at laboratory, fish were placed in a holding tanks (120 cm * 60 cm * 15 cm) with an open sea-water circulation system, receiving a constant flow of sea-water with low level of filtering, which led fish feed. During the experiment the artificial light simulated the natural photoperiod 15h light: 9h darkness (LD), with the lights onset at 05:30 h and lights offset at 21:50 h. Fish were reared to acclimate to laboratory conditions for 4 days prior to the experiment. This research was approved by the Animal Care and Use Committee of the University of Washington.

Experimental design

Experiment 1: Daily activity.

Locomotor activity of 2 sets of 6 fish was recorded in an experimental tank (110.0 cm x 110.0 cm x 60.0 cm), which contained six white boxes (34.0 cm x 31.0 cm x 14.0 cm; 10.7 L) modified via overflow to allow a continuous exchange of water. The monitoring of these 2 sets of fish was performing at two opposite moments of tidal cycle (high and low tide) in nature to check possible differences in sculpin locomotor activity. All keeping conditions were identical to the holding tanks. One fish was placed in each box 5 h before the beginning of the 24 h recording. Two different cameras, one for the day recordings (Logitec, C920 full HD 1080p, Switzerland) and another for the night recordings (Sony, handycam CCD-tr940 XR hi8 8mm Video camcorder camera Player VCR Nightshot, Japan), were both placed 183.0 cm and 174.0 cm above the tank. A Python (Python 2.7, OpenCV 2.4) script was made to record images at one frame per second and track the fish, giving back the coordinates of the fish position every second for each fish. Thus, the swimming distance was determined for the whole 24 hours period.

Experiment 2: Daily variations in escape response.

To study the escape response, a total of 105 Sculpin were tested (each fish only once) every 6 h along a 24 h cycle (01:30, 07:30, 13:30 and 19:30 h). Individual fish were placed in one of 2 experimental 90 L tanks (88.0 x 57.0 x 30.0 cm) (Figure 1A and 1B). Temperature was maintained at 14.5 °C ± 0.5 °C. Sculpin escape responses were provoked by a mechanical stimulus. The stimulus (Figure 1C), a plastic 50 mL centrifuge tube (diameter 3.0 cm; length 12.5 cm; and weight 173.5 g) was held above the tank by an electromagnet and fell through a plastic tube (diameter 10.0 cm; length 120.0 cm), lower edge positioned 5.0 mm above the water surface). A mirror was placed close to the tube to making it possible to identify the first contact of the stimulus with the water surface in the recordings. To test fish escape response during the 3 points in light (07:30, 13:30 and 19:30 h), a high-speed camera (Casio, excilim CMOS shift stabilization EX-FH100, Japan) was placed 80.0 cm above each tank, which recorded at 240 hz. For the nocturnal point (01:30 h), another camera (Sony, handycam DCR-HC96 3.0 megapixels Carl Zeiss Vario-Sonnar T*, Japan) were placed 80.0 cm above a tank. Infrared light was added to permit the recording in total darkness by means of two LED infrared illuminators (Speco, Provideo IR200, USA; and IR Illuminator YY Trade Inc, YY-IR30, USA). Recording were done at 30 hz, and video sequences later deinterlaced and upsampled to 30 hz via half field splitting using (JES Deinterlacer v.3.8.4 for Machintosh, USA).

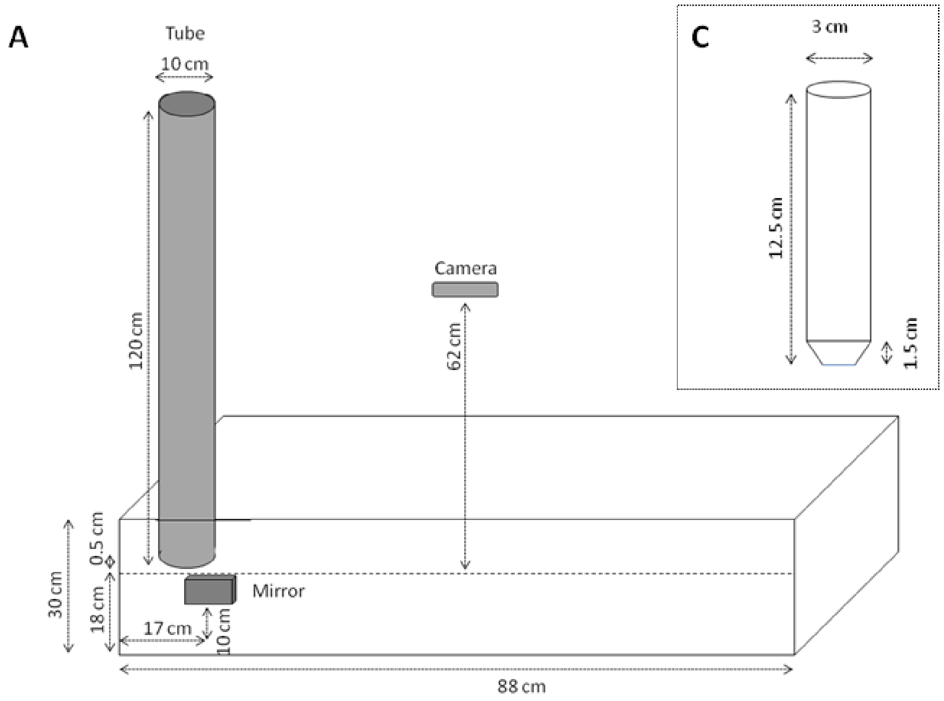
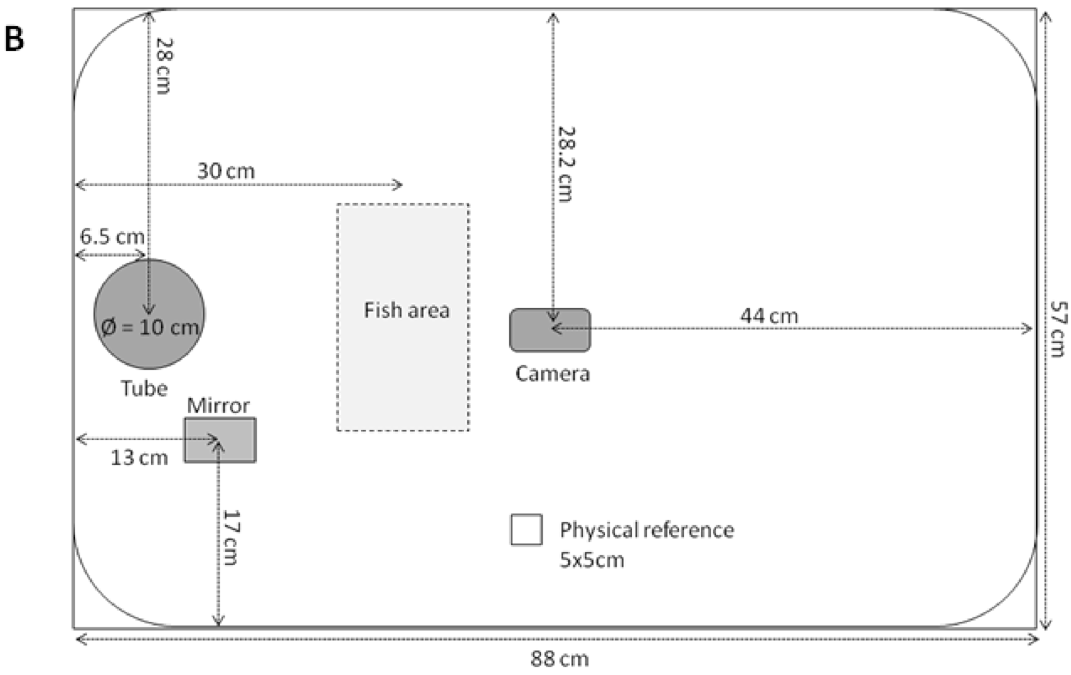


Figure 1. *Diagram of the escape response aquarium and the arrangement of their components, (A) front elevation view, (B) top view and (C) a detail of the stimulus view (Continue).*



The following measurements of escape responses were analyzed:

- Responsiveness, as the proportion of fish that responded to the stimulus within 171.0 ms.
- Latency, as the time between the frame after the stimulus onset and the first frame where fish moved to escape, both included. See Figure 2A and Formula (1).
- Directionality, as AWAY or TOWARDS escape responses correspond to the orientation of the fish C-bend relative to the stimulus (Blaxter et al., 1981).
- Distance travelled by the center of mass of the fish in 83.3 ms (i.e. 5 frames at 60 hz for night-vision camera and 20 frames at 24 hz for the high speed camera) from first movement of the escape response. See Figure 2B and Formula (2).
- Turning angle, as the angle formed by the line through center of mass and the tip of the head of the fish between the first and the last point of the stage 1.
- Average turning rate during stage 1 was calculated using formula (3). See Figure 2C for details.

Latency time in ms, T_L, was calculated on the basis of counted frames, using the formula (1), where f_n is the frame count and and f_r the frame rate in hertz.

$$(1) \quad T_L = f_n \cdot \frac{1000\ [ms/s]}{f_r}$$

Absolute distance travelled, displacement (d) in 2 dimensions (X,Y), was calculated using the formula (2), where (x_1, y_1) indicate the position of center of mass of fish at the frame where the fist fish movement was observed, while (x_2, y_2) indicate its position after 83.3 ms (i.e. 5 frames at 60 hz for night-vision camera and 20 frames at 24 hz for the the high speed camera).

$$(2) \quad d = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$$

Average turning rate, r_a, in stage 1 of the escape response was calculated by the formula (3), where Θ is the angle between the horizontal physical reference line of the escape response tanks and the line through center of mass and the tip of the head of the fish. The time, t, is from first and last point of stage1.

$$(3) \quad r_a = \frac{\Delta\theta}{\Delta t}$$

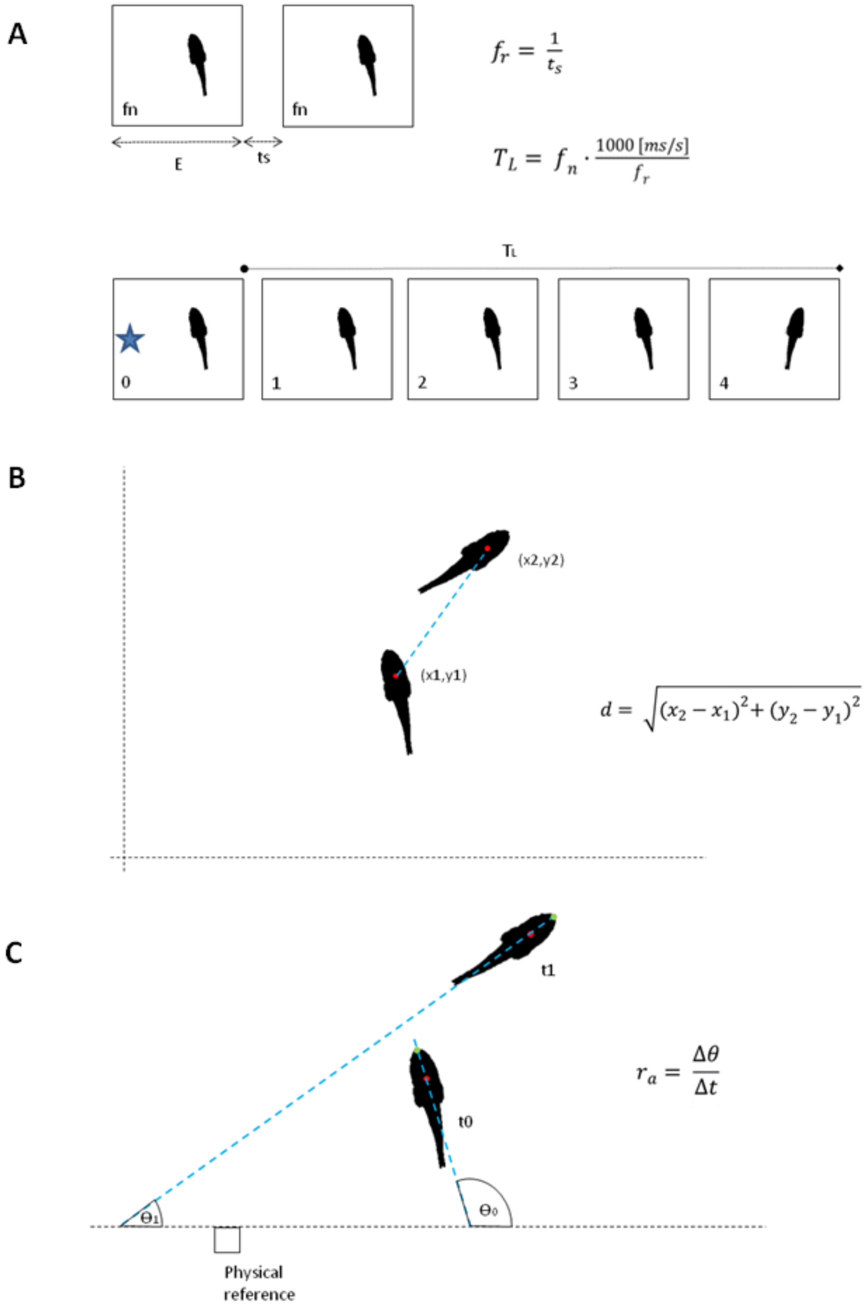


Figure 2. *Chart showing the calculation of (A) escape latency time, T_L, (B) distance travelled, d, at 83.3 ms and (C) turning rate r_a, in stage 1.*

Statistics analysis

A total of 105 fish were tested (i.e. 34 at 01:30 h, 24 at 07:30 h, 22 at 13:30 h and 22 at 19:30 h). Only 47 of these fish responded (i.e. 7 at 01:30 h, 12 at 07:30 h, 14 at 13:30 h and 14 at 19:30 h) and were used for further analysis to test the time of day on escape responses.

Possible differences between sculpin locomotor activity during high and low tides were analyzed by Student t-test converting the percentage data by arcsin funtion. The effects of the time of day on responsiveness and the percentage of turning angles broader than 118° were analyzed using a Chi-squared test, as the corresponding contingency table which had an expected frequency less than five in more than one fifth of its cells (Zar, 1984). One way ANOVA followed by a Tukey test was used to analyze the possible differences in swimming performances (i.e. escape latency, travelled distance, turning angle and turning rate) between different times of day. All these statistical tests were carried out with the SPSS v15.0 program (SPSS Inc., USA) and the significant threshold was fixed in $p < 0.05$. The data are expressed as mean ± Standard Error of Mean (SEM).

RESULTS

Experiment 1: Daily activity.

Sculpin under a simulated natural photoperiod of LD 15:9 h displayed a higher mean daily activity at night (Figure 3), with 82.5 % ± 8.7 % (mean ± SEM) of total daily activity occurring during the dark phase. All observed fish showed nocturnal activity (more than 40% of their daily activity occurred at night). No differences between the daily locomotor rhythm meanwhile there were both high and low tides in nature were found (Student t-test, $p < 0.05$).

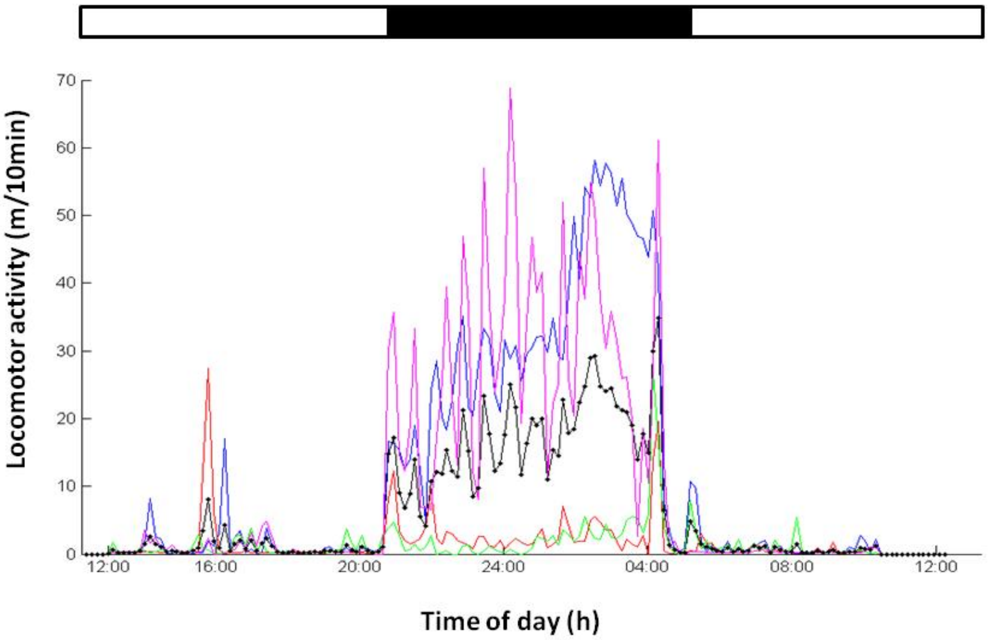


Figure 3. *Profile of locomotor activity of sculpin under a photoperiod of 15 h Light: 9 h Darkness. The vertical axis shows the locomotor activity in meters per 10 minutes and the horizontal axis represents the time of day in hous. Different line colors indicate the activity of different fish, being the black line the mean activity of all fish. White and dark bars on the top represent the light and dark phase, respectively.*

Experiment 2: Daily variations in escape response.

Time of day influenced the percentage of fast escape responses (Figure 4), so sculpin showed the lowest responsiveness (20.6% of fish showed escape responses) in the nocturnal time point at 01:30 h, while during the daytime they displayed a higher responsiveness 50.0% at 07:30 h and 63.2% at both 13:30 h and 19:30 h (Chi-squared, $p < 0.01$).

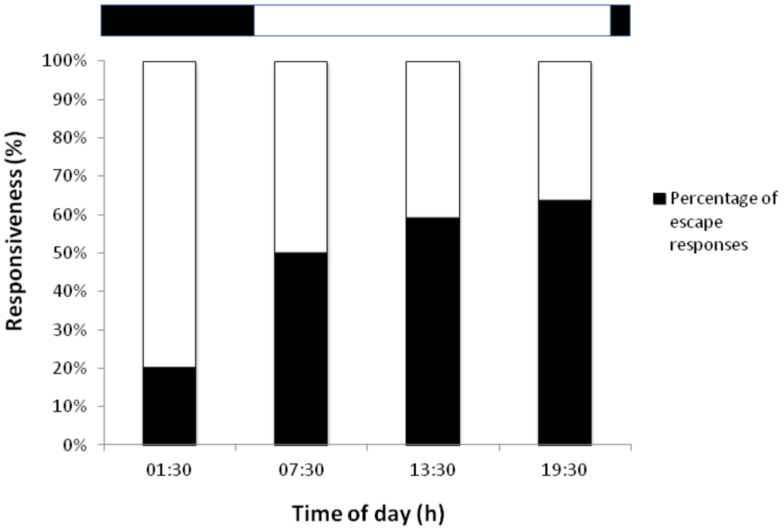


Figure 4. *Daily variation of responsiveness of sculpin. The vertical axis shows the percentage of sculpin escape responses, while the horizontal axis represents the time of day in hours. White and dark bars on the top represent the light and dark phase, respectively. Chi-squared test indicates these data are statistically significant (p < 0.01).*

All fish which presented escape response moved away from the mechanical stimulus, so no differences in directionality were observed. Among these fast escape responses, sculpin travelled dissimilar distances between different times of day (one way ANOVA, $p < 0.01$), covering longer distance (26.40 ± 2.79 cm, mean ± SEM) at 01:30 h than at 07:30 h (17.67 ± 2.79 cm), at 13:30 h (16.20 ± 0.76 cm) and at 19:30 h (16.42 ± 1.06 cm) (Figure 5A). However, other swimming performance parameters, such as turning rate (1.87 ± 0.43 °/ms, mean ± SEM, see Figure 5B) and escape latency (68.77 ± 30.39 ms, mean ± SEM, see Figure 5C) did not statistically differed between times of day (one way ANOVA, $p = 0.24$ and $p = 0.26$, respectively).

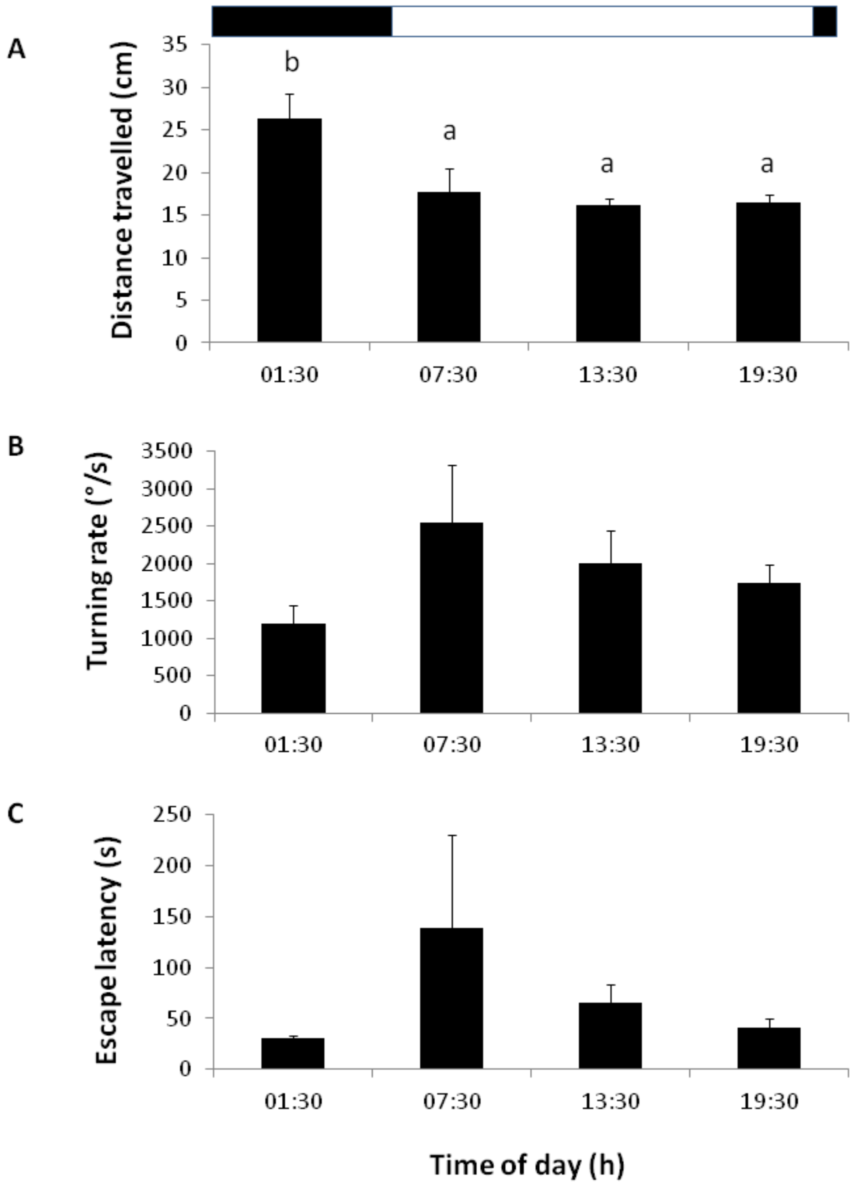


Figure 5. *Mean of distance travelled (A) in centimeters, (B) turning rate in grades per second and (C) escape latency in seconds at 4 different time of day. The horizontal axis shows the time of day in hours. White and dark bars on the top represent the light and dark phase, respectively. Error bars represent the standard error about the mean. Different letters denote significant differences between time points by ANOVA I, p <0.01, followed by Tukey test.*

In general, the turning angle in the escape response covered from 12.69° to 154.62°, never reaching the 180° at any time of day. Particularly as regards each time point, the broadest maximum turning angle (154.62°) was observed at 07:30 h, followed by 147.62° at 13:30 h, 117.26° at 19:30 h and 116.69° at 01:30 h (Figure 6A). Thus, the percentage of turning angle broader than 118.00° was 0.0% at 19:30 h and 01:30 h, 20.0% at 07:30 h and 30.8% at 13:30 h, however, no differences (Chi-squared, $p > 0.05$) were found in the percentage of turning angle broader than 118.00° at different time of day (Figure 6B).

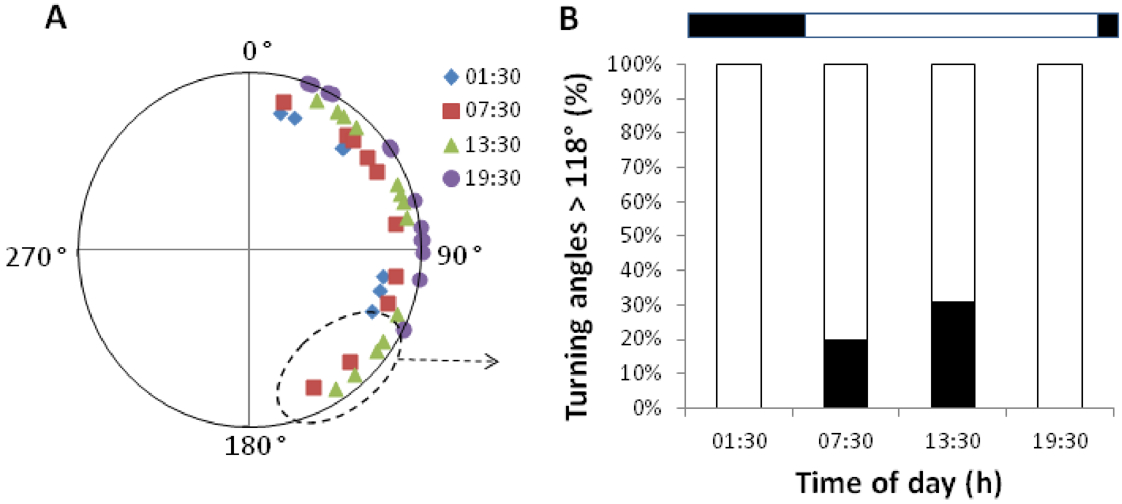


Figure 6. *Turning angles in the escape responses of sculpin at different time of day. (A) The circumference indicates all the possible angles (in degrees) for the escape responses from the stimulus, which corresponds to 0°. Different colors denote different time points. (B) The percentage of turning angles broader than 118° at every time point. The horizontal axis shows the time of day in hours. White and dark bars at the top indicate the light and dark phase, respectively. No significant differences in these percentages between times of day were found by Chi-squared* **test.**

DISCUSSION

Pacific staghorn sculpin (*Leptocottus armatus*) displayed a nocturnal rhythm of locomotor activity under a simulated natural photoperiod of LD 15:9 h and meanwhile there were both high and low tides in nature. Moreover, the responsiveness of escape response (the proportion of fish responding to the stimulus) and the travelled distance in the escape responses varied depending on the time of day, while other swimming performances of the escape response, such as directionality, escape latency and turning rate, did not differ between different times of day.

Light/darkness cycles have been considered the most important synchronizer of biological rhythms in nature. Thus, animals adjust their locomotor behavior to day or night, but rarely throughout 24 h, so being mainly classified as diurnal, nocturnal or crepuscular (Madrid et al., 2001). In accordance with that, sculpin showed a nocturnal mean locomotor activity in the present study, extending the list of nocturnal teleost, such as Senegalese sole (*Solea senegalensis*), Tench (*Tinca tinca)* and European catfish (*Silurus glanis*) (Boujard, 1995; Herrero et al., 2003; Bayarri et al., 2004; Herrero et al., 2005; Boluda Navarro et al., 2009). In this experiment, although all sculpin showed a clear nocturnal locomotor activity, the percentage of nocturnal locomotor activity varied between fish, it is due to fish present a highly flexible circadian system, even existing individuals with different behavioral patterns within the same species (Lopez-Olmeda & Sanchez-Vazquez, 2010).

Despite escape responses are important behavioral processes since they allow fish to avoid predation, up to now, the research in sculpin escape response has been limited to trajectories and distance travelled (Serena et al., 2009; Paglianti and Domenici, 2006). According to the circadian regulation of visual sensitivity of zebrafish which affected the responsiveness of escape responses (Li and Dowling, 1998), in this study, sculpin escape response presented daily variations faced with a mechanical stimulus. The responsiveness of escape response was lower at night than during the daytime, while the travelled distance was higher at night. Responsiveness (together with reaction distance) is the most context-dependent variable component of the escape response due to its presumed strong determination by the relative cost and benefits of escaping, being engaging in other activities (e.g. feeding) (Domenici, 2010). Therefore, time of day extend the list of several environmental factors which have been reported to influence the responsiveness of several fish species, including temperature (Preuss and Flaber, 2003; Szabo et al., 2008), oxygen level (Lefrançois et al., 2005; Lefrançois and Domenici, 2006), turbidity (Meager et al., 2006) as well as mercury and ammonia pollutants (Weber, 2006; Mckenzie et al., 2009).

As regards the context-dependent variability studied by Domenici (2010), our findings about sculpin escape response could be explained by both ultimate (low perception of risk due to adaptive plasticity) and proximate (limitations in the sensory-motor system) hypotheses. Thus, the lowest responsiveness in sculpin escape response at night could be due to ultimate hypothesis since darkness could limit the sensorial capacity of fish to detect the mechanical stimulus by Maunther cells, which receive context-information by visual and mechanoacoustic inputs (Eaton et al, 2001). On the other hand, regarding an ultimate hypothesis, sculpin could perceive lower predation risk during their activity phase (night), and so, their responsiveness of escape response was lower at night although when fish reacted to the stimulus they were more active to travel a longer distance than during the daytime.

In contrast, other swimming performance parameters of the sculpin escape response (such as directionality, escape latency and turning rate) were not affected by the time of day. In this experiment, the directionality of the escape responses was always away, independent of the time of day. Although previous studies reported some environmental factors (i.e. temperature and oxygen) provoked changes in directionality of escape response (Preuss and Faber, 2003; Szabo et al., 2008; Lefrançois et al., 2005; Lefrançois and Domenici, 2006), the away escape response is the more frequent than the toward response, due to the activation of Mauthner cell in the stimulus side, what a contralateral muscular contraction and the away response (Domenici, 2009), and particularly in fish stimulated sideways (instead of head or tail on) (Domenici and Blake, 1993) or single fish (Domenici and Batty, 1997). In spite of our results indicate time of day did not affected sculpin turning rate or turning angle, the broadest maximum turning angle (154.62°), which appeared at early morning and midday (i.e. 07:30 h and 13:30 h), did not reach the theoretical maximum (180°). The sculpin escape latency did not significantly vary in accordance to time of day, similarly to hypoxia did not modify the escape latency in other species (Domenici et al., 2007b), although it was affected by other environmental factors, such as temperature (Webb, 1978; Preuss and Faber, 2003) or pollutants (Mckenzie et al., 2009; Faucher et al., 2006; Weber, 2006).

In summary, the nocturnal locomotor activity of sculpin together with the lowest responsiveness of escape responses and longest travelled distance at night suggested the elusiveness of sculpin is more reduced during their activity phase, but if they then respond to the stimulus, their fast-start performance is greater in terms of travelled distance. It could be due to a sensorial (visual) capacity limited of sculpin Maunther cells in darkness and/or a lower sense of danger but more effective escape response during their activity phase.

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REFERENCES

Batty RS (1989). Escape responses of herring larvae to visual stimuli. J Mar Biol Assoc UK 69: 647–654.

Bayarri MJ, Munoz-Cueto JA, Lopez-Olmeda JF, Vera LM, de Lama M, Madrid JA, Sanchez-Vazquez FJ (2004). Daily locomotor activity and melatonin rhythms in Senegal sole (*Solea senegalensis*). Physiol Behav 81(4): 577-583.

Beddow TA, Van Leeuwen JL, Johnston IA (1995). Swimming kinematics of fast starts are altered by temperature acclimation in the marine fish *Myoxocephalus scorpius*. J Exp Biol 198: 203–208.

Blaxter J HS, Gray JAB, Denton EJ (1981). Sound and startle responses in herring shoals. J Mar Biol Ass UK 61: 851–869.

Boluda Navarro D, Rubio VC, Luz RK, Madrid JA, Sánchez-Vázquez FJ (2009). Daily feeding rhythms of Senegalese sole under laboratory and farming conditions using self-feeding systems. Aquaculture 291:130–135.

Boujard T (1995). Diel rhythms of feeding-activity in the european catfish, *Silurus glanis*. Physiol Behav 58(4): 641-645.

Dill LM (1974). The escape response of zebra danio (*Brachydanio rerio*). I. The stimulus for escape. Anim Behav 22: 710–721.

Domenici P (2002). Context-Dependent Variability in the Components of Fish Escape Response: Integrating Locomotor Performance and Behavior. J Exp Zool 313A: 59–79.

Domenici P (2009). Escape responses in fish: kinematics, performance and behavior. In: Domenici P, Kapoor BG (eds). Fish locomotion: an ecoethological perspective. Enfield, NH: Science Publishers. pp. 123-170.

Domenici P (2010). Context-dependent variability in the components of fish escape response: Integrating locomotor performance and behavior. J Exp Zool A 13: 59–79.

Domenici P, Batty RS (1997). Escape behaviour of solitary herring (*Clupea harengus*) and comparisons with schooling individuals. Mar Biol 128: 29–38.

Domenici P, Blake RW (1993). Escape trajectories in angelfish (*Pterophyllum eimekei*). J Exp Biol 177: 253–272.

Domenici P, Claireaux G, McKenzie DJ (2007a). Environmental constraints upon locomotion and predator-prey interactions in aquatic organisms: an introduction. Philos T R Soc B 362: 1929–1936.

Domenici P, Lefrançois C, Shingles A (2007b). Hypoxia and the antipredator behaviour of fishes. Philos Trans R Soc B 362: 2105–2121.

Eaton RC, Hackett JT (1984). The role of Mauthner cells in fast-starts involving escape in teleost fish. In: Eaton RC (ed). Neural Mechanisms of Startle Behavior. New York: Plenum Press. pp. 213–266.

Eaton RC, Lee RKK, Foreman MB (2001). The Mauthner cell and other identified neurons of the brainstem escape network of fish. Prog Neurobiol 63: 467–485.

Faucher K, Fichet D, Miramand P, Lagardere JP (2006). Impact of acute cadmium exposure on the trunk lateral line neuromasts and consequences on the ‘‘C-start’’ response behaviour of the sea bass (*Dicentrarchus labrax* L.; Teleostei, Moronidae). Aquat Toxicol 76: 278–294.

Fuiman LA, Smith ME, Malley VN (1999). Ontogeny of routine swimming speed and startle responses in red drum, with a comparison of responses to acoustic and visual stimuli. J Fish Biol 55 A: 215–226.

Hale ME (2002). S- and C-start escape responses of the muskellunge (*Esox masquinongy*) require alternative neuromotor mechanisms. J Exp Biol 205: 2005–2016.

Harper DG, Blake RW (1991). Prey capture and the fast-start performance of northern pike Esox lucius. J Exp Biol 155: 175–192.

Huntingford FA, Wright FPI, Tierney JF (1994). Adaptive variation in anti-predator behaviour. In: Bell MA, Foster SE (eds). The evolutionary biology of sticklebacks. Oxford: Oxford University Press. pp. 277–296.

Herrero MJ, Madrid JA, Sanchez-Vazquez FJ (2003). Entrainment to light of circadian activity rhythms in tench (*Tinca tinca*). Chronobiol Int 20(6): 1001-1017.

Herrero MJ, Pascual M, Madrid JA, Sánchez-Vázquez FJ (2005). Demand-feeding rhythms and feeding-entrainment of locomotor activity rhythms in tench (*Tinca tinca*). Physiol Behav 84: 595–605.

LeFrançois C, Domenici P (2006). Locomotor kinematics and behavior in the escape response of European sea bass, *Dicentrarchus labrax* L., exposed to hypoxia. Mar Biol 149: 969–977.

Lefrançois C, Shingles A, Domenici P (2005). The effect of hypoxia on locomotor performance and behaviour during escape in *Liza aurata*. J Fish Biol 67: 1711–1729.

Li L, Dowling JE (1998). Zebrafish visual sensitivity is regulated by a circadian clock. Visual Neurosci 15: 851–857.

Lopez-Olmeda JF, Sanchez-Vazquez FJ (2010). Feeding rhythms in fish: from behavioral to molecular approach. In Kulczykowska E (ed.). Biological clock in fish. Enfield, NH: Science Publishers. pp. 155–184.

Madrid JA, Boujard T, Sanchez-Vazquez FJ (2001). Feeding rhythms. In: Food intake in fish, Houlihan DF, Boujard T, Jobling M (eds.). Blackwell Science, Oxford. pp. 189–215.

Markel RW (1994). An adaptive value of spatial-learning and memory in the blackeye goby, *Coryphopterus-nicholsi*. Anim Behav 47: 1462–1464.

McKenzie DJ, Shingles A, Claireaux G, Domenici P (2009). Sublethal concentrations of ammonia impair performance of the teleost faststart escape response. Physiol Biochem Zool 82: 353–362.

Meager JJ, Domenici P, Shingles A, Utne-Palm AC (2006). Escape responses in juvenile Atlantic cod *Gadus morhua* L.: the effects of turbidity and predator speed. J Exp Biol 209: 4174–4184.

Paglianti A, Domenici P (2006). The effect of size on the timing of visually mediated escape behaviour in staghorn sculpin Leptocottus armatus. J Fish Biol 68: 1177–1191.

Preuss T, Faber DS (2003). Central cellular mechanisms underlying temperature-dependent changes in the goldfish startle-escape behavior. J Neurosci 23: 5617–5626.

Serena G, Banet AI, Domenici P (2009). Do nearby obstacles modify escape response? A preliminary investigation in Pacific Staghorn Sculpin (*Leptocottus armatus*). Comp Biochem Physiol A 153: S131–S132.

Szabo TM, Brookings T, Preuss T, Faber DS (2008). Effects of temperature acclimation on a central neural circuit and its behavioral output. J Neurophysiol 100: 2997–3008.

Wardle CS (1975). Limits of fish swimming speed. Nature (London) 255: 725–727.

Webb PW (1976). The effect of size on the fast-start performance of rainbow trout Salmo gairdneri and a consideration of piscivorous predator-prey interaction. J Exp Biol 65: 157–177.

Webb PW (1978). Temperature effects on acceleration of rainbow trout *Salmo gairdneri*. J Fish Res Board Can 35: 1417–1422.

Webb PW, Zhang H (1994). The relationship between responsiveness and elusiveness of heat-shocked goldfish (*Carassius auratus*) to attacks by rainbow trout (*Oncorhynchus mykiss*). Can J Zool 72: 423–426.

Weber DN (2006). Dose-dependent effects of developmental mercury exposure on C-start escape responses of larval zebrafish *Danio rerio*. J Fish Biol 69:75–94.

Weihs D (1973). The mechanism of rapid starting of slender fish. Biorheology 10: 343–350.

Weihs D, Webb PW (1984). Optimal avoidance and evasion tactics in predator-prey interactions. J Theor Biol 106: 189–206.

Zar JH (1984). Statistical significance of mutation frequencies, and the power of statistical testing, using the poisson-distribution. Biometrical J 26(1): 83—88.