Behavioral Responses of Javanese Medaka (Oryzias Javanicus) Versus Zebrafish (Danio Rerio) in Open Field Test.

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Abstract

Background: Locomotion is integral for animal survivability. However, the understandings of locomotor that lead to exhibition of multiple complex behaviors of fish models in response to an open field environment still remain unresolved. To determine whether two different fish models, Javanese medaka and zebrafish have similar baseline locomotor activity in open field paradigm, an open field test was used.

Results: Results showed that Javanese medaka exhibit increased in exploratory activity with lower anxiety responses; exhibit a steady habituation response in OFT paradigm and vice versa in zebrafish. Medaka also took longer duration to establish home-base in comparison to the zebrafish. Although no other motor responses were observed, both fish species displayed strong preference of left eye used to assess the OFT tank.

Conclusion: Medaka exhibits slower locomotors activity, lower anxiety responses and steadily maintains its locomotion once they reached habituation. In comparison, zebrafish demonstrated bolder behavioral phenotypes where they showed faster locomotors activity, higher anxiety responses with similar habituation response to the Javanese medaka. Thus, this present study revealed that two different teleost aquatic model organisms, Javanese medaka and zebrafish have different behavioral phenotypes in open field test.

Background

Locomotion is integral for animal survivability to navigate foods, shelters and evade predators (Kiehn and Dougherty, 2016). Diverse modes of locomotion regulated by homologous circuit of central nervous system in vertebrates have been well documented in numerous species for centuries (Berg, et al., 2018). Due to their small size, short life cycle, amenability to genetic manipulation as well as complex behavioral repertoire, two teleost fishes namely, Javanese medaka (*Oryzias javanicus*) and zebrafish (*Danio rerio*) are ideal experimental models for studying locomotor and complex neuropsychological behavior of vertebrates. However, the baseline of locomotor behaviors of both fish models in an open field environment still remain poorly understood. Although Open Field Test (OFT) was originally developed in 1934 as a test to evaluate emotionality in rodents, the use of this behavioral paradigm has extended to wide-range of animal species such as primates, calves, pigs, rabbits, lobsters, and honeybees (Hall, 1934; Prut and Belzung, 2003). Currently, this paradigm is widely used in psychology, neuroscience and various research fields in order to understand animal's locomotor behavior across a specific range of time in response to drugs or chemicals delivery (Seibeenhner and Wooten, 2015; Mi et al., 2016; Kraeuter et al., 2019). The factors contributing to the attainment of OFT as one of the most widely used measures of behavior in animal psychology includes its rapid evaluation of well-defined behaviors, easy, requires no training to the test subject, and minimal specialized training for the experimenter. As zebrafish has become one of the most preferable aquatic vertebrate for neuroscience research (Kalueff et al., 2014; Sakai et al., 2018; Saleem and Kannan, 2018), various types of rodent behavioral paradigms have been adapted to suit the nature of fish to be in the water, including OFT (Matsunaga and Watanabe, 2010; Baker et al., 2018).

OFT usually provides an index of general behavior in particular exploratory activity, which is a crucial response to novelty (Lynn and Brown, 2009; Rosemberg et al., 2011). Like rodents, zebrafish exhibit thigmotaxis, as initial response to OFT and increased exploratory activity, which spikily decreases during the trial, reflecting habituation had achieved (Leussis and Bolivar, 2006; Kysil et al., 2017). Thigmotaxis is preference of animals towards the periphery of an open field arena and avoiding center area which ideally used to evaluate anxiety status (Schnörr et al., 2012). Animals tend to establish a reference place (home-base) which they return regularly after exploring the environment and spent more time during trial (Rosemberg et al., 2011; Stewart et al., 2012). Home-base formation has been reported to reflect a behavioral state comparable to thigmotaxis (Stewart et al., 2012). Mounting evidence shows that zebrafish display robust anxiety-like behavior in open field-based paradigms (Singer et al., 2016; Nema et al., 2016; Hong and Zha, 2019). Moreover, a novel environment may induce aggression behavior in animal where zebrafish shows aggression by displaying different type of specific behaviors such as charges with bites and chases, circling, and establishing dominance (Kalueff et al., 2014; Zabegalov et al., 2019). Hence, OFT test emerges as an interesting tool to reveal the animal's interaction in a novel environment that offers valuable and reliable behavioral assessment to explain comprehensive sequential (spatio-temporal) structure of the exploratory behavior (Macrì et al., 2017).

Javanese medaka (*Oryzias javanicus* Bleeker, 1854) is a small tropical fish that exhibit similar phenotypes to the established laboratory fishes and have potential to be used as model organism for neuroscience research (Imai et al., 2007). It has a wide geographical range including Malaysia, Indonesia, Western Borneo, Singapore and Thailand. Recently, Javanese medaka has been proposed as a model organism for biological, ecological and ecotoxicological studies (Aziz et al., 2017; Ferdaus et al., 2018; Amal et al., 2019). The comparable versatility of Javanese medaka with zebrafish such as its ability to occupy fresh, brackish and saltwater, perennially available, rapid growth rate, short life cycle and easy maintenance in the laboratory (Inoue and Takei, 2002), urge the need to improve current understanding about their baseline behavior in response to an open field environment. The generated knowledge will serve as a starting point in the attempt to predict their behavioral responses upon exposure to different types of xenobiotic compounds. Complex behaviors such as anxiety, habituation and learning (Nema et al., 2016; Kysil et al., 2017; Blaser and Vira, 2014) exhibit by the fish model offering a unique translational opportunity to model brain disorders (Champagne et al.,2010; Panula et al., 2010). Well-developed sensory motor and cognitive systems, high sensitivity to environmental stimuli, and quantifiable behavioral phenotype highlights their strengths as emerging models for neurobehavioral research (Signore et al., 2009; Cachat et al., 2011; Kalueff et al., 2014). Thus, this will help to improve our fundamental understanding about their neurobehavioral responses before further manipulation in more advanced studies. Therefore, the aim of the current study was to compare behavioral responses of Javanese medaka (*Oryzias javanicus*) in comparison to zebrafish (*Danio rerio*) upon introduction into OFT. Specifically, this study assess locomotor, habituation, exploratory, thigmotaxis and other motor responses for each fish models in the first six minutes in the OFT paradigm.

Materials And Methods

Animal husbandry

All experiments were performed in accordance with the rule of Institutional Animal Care and Use Committee of Universiti Putra Malaysia (UPM) (UPM/IACUC/AUP-R049/2019). Wild-type short fin adult zebrafish (*Danio rerio*) of mixed sex were purchased from local petshop (AquaMart, Kajang) and retained at Laboratory of Natural Products, Institute of Bioscience, UPM. The fish were maintained in aquarium water (de-chlorinated, UV treated, aged tap water) at 28°C, fed with brine shrimp (*Artemia salinaa*) four times daily and kept in a standard 14/10 hour light/dark cycle (Koerber and Kalishman, 2009). Adult Javanese medaka (*Oryzias javanicus*) were collected from an estuary located in Sungai Pelek, Sepang, Selangor (2.6416° N, 101.7148° E). Then, the fish were acclimatized to a freshwater environment by gradual reduction of salinity from 18.50 to 0.00 ppt in the same laboratory within two weeks (Aziz et al., 2017). Both fish species (n=30; size 3-5 cm) were acclimatized for 10 days in the laboratory condition in 40L top-filtered tank (61 cm length X 31 cm width X 31 cm height). Then, behavioral evaluations in OFT for both types of fish were conducted in a separate behavior room. Following behavioral recording, the fishes were euthanized using 500 mg/L Tricane (Sigma-Aldrich, St.Louis, MO) for approximately 10 mins until there were no operculum movements observed (Strykowski and Schech, 2015).

Open field test (OFT) setup

Open-field test behavioral recordings were performed between 8.00 a.m and 2 p.m in a behavioral recording room under standard illumination (~1000-1100 lux). 24 hour prior to the behavioral assessment, 10 fishes for each fish species were transferred into holding tanks (30.0 cm length X 16.0 cm width X 18.0 cm height) and were kept in the behavioral recording room. Then, each fish was carefully netted into the open field test (OFT) tank for behavioral recording to evaluate the locomotor activity, thigmotaxis, home-base formation and other motor responses. Fish handling and all behavioral recording procedures were conducted by a single observer blinded to any treatment of the fish.

The fish behaviors in the OFT tank were recorded in a customized closed framework as shown in the Figure 1A for 6 minutes (modified from Nema et al., 2016). The closed framework was used to eliminate the influence of outside interferences like the presence of experimenter from affecting the behavior of the fish. The behaviors of the fishes were recorded for six mins, using a 18-megapixels DSLR (1200D, Canon Inc., Japan) equipped with 55 mm kit lens (set to video setting, with manual focus) that was placed at the top center of the tank at the distance of 90 cm from the bottom of the tank. A white corrugated plastic sheet was used to surround a customized light box (± 1000 lux) (55 cm length X 55 cm width X 10 cm height), in order to ensure uniform light distribution. The OFT tank (37 cm length X 29 cm width X 18 cm height) filled with aquarium water was placed on the top of the light box. At the end of the behavioral recording, the fish was removed from OFT tank for euthanization. OFT tank was rinsed and filled with new aquarium water before subsequent recording. The behavioral recordings for both types of fish species (n=30) were conducted on different day within the same week.

Locomotor behavior

The behavioral analysis was conducted offline in a computer using ZebraLab 4.2 software (ViewPoint Life Sciences, France) to track the locomotor activity of the fish for 30 frame per sec (Crispim Junior et al., 2012; Audira et al.,2019). The video-tracking data were then used to evaluate relevant measures of exploration and habituation across time such as total distance moved and speed travelled. The total distance moved and speeds travelled for six mins trial were representing the total locomotor activity. To identify the exhibition of exploratory and habituation response, both total distance moved and speed travelled were divided into three phases such as early, mid and final with two mins intervals for each time interval. Other endpoints that were measured during the test including time spent per area (center/middle), number of entries to the center, inactive count at the center and inactive duration at the center.

Thigmotaxis

Thigmotaxis or wall hugging is the preference of animal to avoid center of arena and move in closer to the edge of an open field arena which used as anxiety index (Schnörr et al., 2012). For thigmotaxis data acquisition, the recorded videos were analyzed using ZebraLab 4.2 software (ViewPoint Life Sciences, France) and the regions of interest were drawn using the software into three main regions: outer, middle and center (Figure 3A). Thigmotaxis was presented as the percentage of the total distance moved at the edge divided by total distance moved multiplying by a factor of 100 (Schnörr et al., 2012). The data were analyzed for every 1, 2 and 3 mins in order to identify the optimal time interval to quantify the thigmotaxis behavior. The analysis was separated based on time interval to prevent the influence of exploratory behavior exhibited by the fish in the early period of recording.

Home-base formation

The home-base is represented as the section where the animals repeatedly return after exploring the surrounding and spent the longest time in the open field arena (Eilam and Golani, 1989; Rosemberg et al., 2011).The trace of fish swimming behavior was generated by video-tracking system to visualize fish locomotor behavior and home-base formation by using ImageJ (https://imagej.nih.gov/ij/) plugin, AnimalTracker API (http://animaltracker.elte.hu/ plugins). This tracker plugin provides the X,Y-coordinates of the fish in each frame by processing the recorded videos (Gulyás et al., 2016). Then, the videos were imported into ImageJ as virtual stacks and converted into 8 bit gray scale. The processing chains of the recorded videos using ImageJ software were based on the previous studies by Gulyás et al., 2016. The X,Y- coordinates were then exported into Microsoft Excel as a text file. The videos with tracking and representative tracking were saved as AVI and JPEG format using ImageJ respectively. The representative tracking with higher number of movement at the edge of the OFT arena were used to represent the home-base formation of two different fish species across time (Rosemberg et al., 2011).

Other motor responses

Other motor responses exhibited by both fish model were also observed in the OFT tank. These responses were manually scored and analyzed by trained observers including: i) percentage of behaviors occurrence towards the OFT walls (dashing, dashing along paradigm, head contact and no dashing), ii) percentage of time spent for body posture orientation towards the OFT wall (left, right and frontal-vertical) and iii) percentage of body movement (clockwise) (Figure 4A) (Kohda et al., 2019). The fish was considered exhibits dashing along when the body postures exposing either right or left side of the head towards the OFT walls. Head contact was considered when the fish orient its body in frontal-vertical postures exposing the head vertically towards the OFT walls. Meanwhile, dashing and stopping was considered when the fish orient its body either right or left to the OFT wall and stay immobile for approximately less than 0.1 sec. No occurrence of dashing as described above was classified as no dashing behavior.

Fish body movement were then determined as clockwise movement if the turn angle is positive value ranging from -180° to 180° (Rosemberg et al., 2011). All the X and Y coordinates obtained through a freeware automated AnimalTracker plugin, ImageJ were then exported and further analyzed using Microsoft Excel based on Equation as suggested by previous study (Nema et al., 2016). The absolute turn angle represents the sum of all vectors movement created from one position to animal's centroid to the next. The turn angle of fish between frames calculated as theta (θ) degree from the slope using Equation. The slope was determined as the ratio of ΔY and Δ X. ΔX and ΔY are the differences between locations of X and Y in successive frames respectively. X_{next}, $X_{previous}$ and Y_{next}, $Y_{previous}$ are successive X and Y frames respectively.

$$\text{Turn angle } (\theta) = \frac{((\tan^{-1}\frac{\Delta y}{\Delta x}) * 180)}{\pi} \qquad \text{Equation}$$

$$\Delta X = X_{next} - X_{previous} \text{ and } \Delta Y = Y_{next} - Y_{previous}$$

Statistical analysis

The statistical analyses were conducted using SPSS (SPSS v.22.0). Two-way analysis of variance (ANOVA) was used to determine the differences of behavioral responses between two adult fish species, Javanese medaka and zebrafish in OFT. *Dunnet test* was set at $p<0.05$ as the threshold for statistical significance to compare behavioral responses between two different fish species and within different time intervals. Data were represented as average values ± standard error of mean (SEM). GraphPad Prism

6.0 version statistical software (GraphPad Software, USA) was used for all graphs. The parameters that were observed include exploratory and habituation response (total distance moved, speed travelled), anxiety-like behavior (percentage of thigmotaxis) and aggressive behaviors [i) percentage of occurrence of behaviors: dashing along the paradigm, head contact with paradigm, dashing and stopping and no dashing; ii) percentage of time spent for different body posture: left, right and frontal vertical, iii) percentage of clockwise body movement].

Results

Spontaneous locomotor activity

Javanese medaka exhibited lower locomotor activity and vice versa for zebrafish over 6 mins behavioral recording using OFT tank. Figure 1B showed the representative swimming tracking of Javanese medaka and zebrafish during 6 mins that was divided into three time intervals. Javanese medaka have lower locomotor activity [total distance moved: $F (35, 19.34) = 38.8131$ cm, $P<0.001$; swimming speed: $F (35, 18.75) = 10.0193$ cm/sec, $P<0.0001$] than zebrafish [total distance moved: $F (35, 19.34) = 133.097$ cm, $P<0.001$; swimming speed: $F (35, 18.75) = 27.2054$ cm/sec, $P<0.0001$] (Figure 1C-D). The total distance moved for Javanese medaka was three times lower than zebrafish while swimming speed was two-fold lower than zebrafish. Even though the total distance moved and swimming speed for Javanese medaka and zebrafish increased and decreased respectively over time, however, there were no overlapping observed throughout the recording time (Figure 1E-F).

Spontaneous locomotor activity was analyzed based on 2 mins time interval where the first 2 mins (early phase) was considered as exploratory phase and the remaining 4 mins (mid and final phase) was considered as habituation responses. During the first 2 mins in the OFT, Javanese medaka displayed an increment of spontaneous locomotor activity with time in contradictions to zebrafish (Figure 1E-F). Javanese medaka demonstrated a gradual increased while zebrafish exhibited a moderate decreased in spontaneous locomotor activity. Both fish species reached a plateau in their locomotion at mid-phase, showing that both fish species started to habituate in the OFT (Figure 1E-F). These findings suggest that Javanese medaka and zebrafish have different baseline locomotor activity in a novel environment.

Thigmotaxis and home-base formation

Thigmotaxis is a tendency of an animal to move in contact with vertical surface and commonly moves towards the periphery of a novel arena avoiding center area. Thigmotaxis was measured as percentage of total distance moved in outer area over total time duration. To compare thigmotaxis in both Javanese medaka and zebrafish in different time frames (every 1-6 mins intervals), two way ANOVA and Dunn-Sidak test were performed. Results showed that thigmotaxis for every 1 mins intervals in Javanese medaka were significantly increased at 240 s (82.65 ± 5.99 %) until 360 s (88.55 ± 5.99 %) (Figure 2A). Meanwhile, thigmotaxis in zebrafish also increased significantly at later time intervals, 300 s (82.51± 5.89

%) until 360 s (80.71 ± 5.89 %). For every cumulative 2 mins intervals, Javanese medaka exhibited a gradual increased in thigmotaxis from 54.696 ± 5.315 % to 88.1796 ± 5.315 %. On the other hand, thigmotaxis percentage of zebrafish increased from 60.19 ± 5.225 % to 81.6066 ± 5.225 % (Figure 2B). Data analysis using cumulative 3 mins intervals for both fish species tested, showed a prominent increment trend in thigmotaxis from 59.8709 ± 5.126 % to 86.3366 ± 5.126 % for Javanese medaka and 64.244 ± 5.083 % to 79.7248 ±5.083 % for zebrafish (Figure 2C). Since both fish species started to display a significant edge preference after 3 mins, therefore, these findings suggest that the most ideal approach to analyze thigmotaxis behavior in OFT could be after 3 mins behavioral recording. Generally, both fish species showed increased percentage of thigmotaxis behavior with time.

Figure 2D-O showed representative swimming tracking in the OFT tank for every one min for both fish species. In the first minute, Javanese medaka remained immobile for at least 23 sec at the edge, and then immediately swam to the center and middle region. After that, Javanese medaka swims towards the edge of the OFT swimming arena (Figure 2D, additional file 1). In comparison to medaka, zebrafish immediately swam towards every edge of the OFT tank within the first min (Figure 2J, additional file 2). Formation of home-base by Javanese medaka (Figure 2F-I) and zebrafish (Figure 2N-O) were observed when they notably showed a prominent preference towards the edge of similar location. A distinct formation of home-base started to form once they were habituated to the OFT tank. The home-base formation was represented by the highest tracking movement formed at the edge of the OFT tank.

Figure 3B showed that Javanese medaka exhibited insignificant number of entries to the center, while zebrafish showed a significant decreased. These results showed that both fish species still utilized the middle and center regions of the swimming arena as horizontal transition to explore the OFT tank. During the first min, Javanese medaka remained inactive for more than 16.53 ± 1.956 sec at the center. In comparison to zebrafish, they spent less than 0.2433 ± 1.956 sec at the center before they immediately swam towards the edge of OFT tank (Figure 3C). Meanwhile, in the first 60 s in OFT, Javanese medaka displays a 30-fold higher number of inactive counts at center region (3.690 ± 0.6520) in comparison to zebrafish (0.1333 ± 0.6520) (Figure 3D). In the subsequent time intervals, Javanese medaka display 2-fold increment of inactive count at 120 s (6.172 ± 0.6520) and decreased over 6 mins time. In contrast, zebrafish displayed almost zero inactive count at the center throughout the 6 mins (0.0 - 0.3333 ± 0.6520). These results suggest that Javanese medaka were less active, calmer and have lower anxiety responses. In comparison, zebrafish were found to be more active, aggressive and have higher anxiety responses.

Other motor responses

To evaluate other motor responses for both type of fishes, different parameters were used including i) dashing along OFT wall, ii) head contact to the OFT wall, iii) dashing and stopping performed alongside the OFT wall and iv) other behaviors (Figure 4A). Javanese medaka exhibited highest occurrence of head contact with the wall of the OFT (46.31 ± 3.832 %), followed by dashing along the wall (42.41 ± 3.832 %), dashing and stopping (5.103 ± 3.832 %) and other behaviors (6.138 ± 3.832 %) (Figure 4B). Meanwhile, zebrafish immediately swam towards the edge of the OFT tank and exhibited dashing along the wall of OFT (43.53 ± 3.832 %), followed by head contact with the wall (38.63 ± 3.832 %), dashing and stopping (9.600 ± 3.832 %) and other behaviors (8.333± 3.832 %) (Figure 4B). Both fish species displayed a balance body posture as they spent left body orientations (Javanese medaka: 46.00 ± 3.030 %; zebrafish: 38.66 ± 3.030 %) and right body orientations (Javanese medaka: 37.64 ± 3.030 %; zebrafish: 40.93± 3.030 %) equally, over 6 mins trial to navigate and explore the OFT tank (Figure 5B). In addition, they also demonstrated a frontal-vertical body posture to the OFT walls where both fish species had no significant different (Javanese medaka: 16.29 ± 3.030 %; zebrafish: 20.45± 3.030 %). Furthermore, this study also recorded fish body movement (clockwise or counterclockwise) to determines which eye (left or right, respectively) was used to view the edge of the OFT wall. Javanese medaka displayed a significant clockwise body orientation at the early phase in comparison to zebrafish (Javanese medaka: 28.03 ± 2.339 %; zebrafish: 20.20 ± 2.339 %). Meanwhile, there were no significant different for both species during mid (Javanese medaka: 26.97 ± 2.339 %; zebrafish: 22.33 ± 2.339 %) and final phase (Javanese medaka : 27.31 ± 2.339 % ; zebrafish: 23.73± 2.339 %) (Figure 4D). No significant differences were observed for anticlockwise orientation in both fish species (Supplementary Figure 1). Thus, this finding may indicate that both species have strong preferences to use their left eyes in assessing a novel environment.

Discussion

This study demonstrates the comparison of locomotor behaviors between two different fish models, Javanese medaka and zebrafish for 6 min using OFT. This assay is crucial in determining whether both fish species have similar baseline activity in an open field-based paradigm. Three different time frames were used to measure the net activity for both fish species. Early phase was analyzed as exploratory behavior while the remaining time, (mid and final phase) were used to evaluate the habituation responses. Results showed that Javanese medaka steadily increased their locomotor activity with lower anxiety responses in a novel-based paradigm. Meanwhile, zebrafish displayed a decreased in locomotor activity during early phase with higher anxiety responses.

Exploration plays an important role in animal’s natural behavior that is mainly used to investigate novel environments and to sustain their survivability (Baker et al., 2018). Interestingly, Javanese medaka took a longer duration before exploring the OFT tank. These findings suggest that Javanese medaka were characterized as passive individuals since they displayed shorter swimming distance and lower exploratory activities. In comparison to Javanese medaka, zebrafish have bolder behavioral phenotypes in open field environment since they rapidly swam throughout the novel arena, thus resulting in longer swimming distance. Boldness and shyness primarily denote the willingness among individual to take risk particularly in novel environment (Sih et al., 2004; Mustafa et al., 2019). The apparent disparity behavior between fish species may have arisen due to variation between domesticated (laboratory) zebrafish and wild Javanese medaka population. This findings corroborate with previous studies where Hutter et al., (2011) found that wild-derived zebrafish were more shy and distressed in captivity, whereas the domesticated zebrafish were regularly bold and eagerly approach human and other stimuli (Wright et al., 2006; Moretz et al., 2007). Besides, the captive environment which was highly stable with the absent of predators likely results in rapid domestication that leads to increase in boldness behavior in all captive vertebrates (Huntingford, 2004; Matsunaga and Watanabe, 2010; Agnvall et al., 2015; Rojas-Carvajal et al., 2018; Mustafa et al., 2019). Noteworthy, the difference in boldness and risk taking behavior of wild-type Javanese medaka versus domesticated zebrafish could also have been influenced by their genetic variation (Wright et al., 2006; Norton et al., 2011). However, further study should be conducted in the future to evaluate the differences of exploratory behavior for domesticated Javanese medaka versus wild caught Javanese medaka. A constant in the locomotor activity over time also implicated that the exploratory activity started to diminish due to exhibition of habituation response (Rankin et al., 2009).

Habituation is defined as a behavioral response decrement which results from repetitive stimulation without involving sensory adaptation/sensory fatigue or motor fatigue (Rankin et al., 2009; Thompson, 2018). Notably, Javanese medaka took slightly longer time to habituate in the OFT tank, while zebrafish habituated faster to the OFT environment. Different rate of habituation within populations are probably shaped by natural selection which influenced by animal’s adaptation towards its natural habitat (Bell and Peeke, 2012). A steady habituation in Javanese medaka might be due to their preferences for shallow periphery of the rivers where water current is slower and away from any point of disturbances (Yusof et al., 2013). In contrast, rapid habituation observed in zebrafish likely due to their natural habitat selection in the wild. They inhabit a wide range of habitats, including still, slow-flowing and fast-flowing water bodies (Suriyampola et al., 2016). Animals that experienced a more complex rearing environment during their development will habituate faster to novelty (Zimmermann et al., 2001). Moreover, this active learning process may help fish to distinguish false alarms to harmless events involving different mechanisms depending on stimulation, sensory pathway, and signal processing which eventually trigger various exploration responses in fish (Raderschall et al., 2011; Godwin et al., 2012; Gaspary et al., 2018). Variations in natural habitat preference and adaptation for different fish species reduce the risk of predation and increase their survivability. Natural habitat variations between two different fish species might influenced their habituations response in the OFT tank.

Thigmotaxis is a preference of animals towards the periphery of a novel arena and avoiding center area which ideally used as anxiety index (Schnörr et al., 2012). In the present study, Javanese medaka were found to be passive and have lower anxiety responses in comparison to zebrafish that were active and more aggressive, with higher anxiety responses. A significant edge preference started to be displayed by both fish species after 3 min, suggesting that the ideal time to analyze thigmotaxis behavior in OFT should be measured starting from fourth mins in OFT. The early phase of behavioral recording in a novel environment is less suitable for thigmotaxis assessment due to the influence of exploratory behavior (Gould et al., 2009). Like human and rodents, anxiety have been linked to specific behavior phenotypes where the organism avoid open spaces, searching for safety in enclosed space as a defensive response, lack of motivation to explore and also sometimes associated with cognitive dysfunction (Walz et al., 2016; Higaki et al., 2018). Since teleost also shared evolutionary conserved neural circuits in regard to anxiety-like behaviors (Belzung and Philippot, 2007; Stewart et al., 2012), both of fish models in this study clearly displayed thigmotaxic behavior. The preference to the periphery proposed that both fish species utilized vertical surfaces of the OFT wall as a spatial clue for navigation. This similarity of behavioral strategies for both fish species and rodents in novel environment suggest that OFT walls serve as a guiding and attractive force on locomotion which modulates their direction and speed in reference to walls during OFT navigation (Horev et al., 2007). These forms of attraction were suggested involving recognition and locational memory which may also underlie the Javanese medaka and zebrafish locomotion found in this study (Stewart et al., 2010). However, anxiety-related neuroendocrine responses should be taken into consideration in the future study by analyzing whole-body cortisol levels (Yeh et al., 2013). This will further confirm whether exposure to open field environment altered anxiety responses in both fish species, physiologically.

Furthermore, this study discovered that the formation of the home-base for both fish species were established at the edge of the OFT. The finding revealed that both fish species have one prominent home-base formation that was commonly established close to the OFT walls. Worth mentioning, Javanese medaka takes slightly longer duration to establish a home-base in comparison to zebrafish. A distinct formation of home-base started to form once they were habituated to the OFT tank. The home-base is denoting as a place in the area for which the experimental animal shows a preference across time, which typically exhibited in open field-based paradigm (Rosemberg et al., 2011; Stewart et al., 2012. Similar type of home-base formation also has been documented in rodent studies (Horev et al., 2007; Thompson et al., 2018).

With respect to other motor responses in OFT, both fish species displayed a balance left and right body orientations in order to navigate and explore OFT tank. No significant different in the percentage of frontal-vertical body posture towards the OFT walls were observed for both fish species. This frontal-vertical body posture suggested that the fish exhibit a pause and look behavior. This behavior is an important aspect of exploration that allows information gathering which required for decision making during their exploration in novel environment (Kalueff et al., 2014; Redish, 2016). It is worth to note that the formation of fish reflection on the OFT wall made up of transparent acrylic sheet during the behavioral recording was observed. This study was accidentally found that zebrafish responded aggressively to its own reflection on the wall of OFT tank in comparison to Javanese medaka. Nonetheless, this study was unable to determine whether the fish can view its own reflection on the acrylic sheet. Therefore, to determine whether the fish have self-recognition, this visually mediated behaviors can be further explored for future research by tracking eye movement using optokinetic reflex (OKR), oculomotor behavior and a built in customized paradigm specialized for self-recognition assessment should be as well implement (Maurer, et al., 2011; Dehmelt et al., 2018).

This study also assessed fish body movement (clockwise or counterclockwise) to determine which eye (left or right, respectively) was used to view the edge of the OFT wall. Both Javanese medaka and zebrafish significantly displayed lower than 50% of clockwise body movement throughout early, mid and final phase, suggesting that both fish species have strong preference to use their left eye. No significant differences were observed for anticlockwise orientation (right eye) in both fish species (additional file 3).

In accordance with the present study, previous studies have reported that adult zebrafish used their left eyes to assess the novelty of objects or environment whereas right eyes were used for prey detection (Miklósi, et al., 1997; Sovrano, 2004; Watkins et al., 2004; Sovrano and Andrew, 2006).

This study showed that 6 min duration for behavioral recording in OFT was sufficient for both Javanese medaka and zebrafish to exhibit a visible thigmotaxis and home-bases behavior. A previous study showed that zebrafish exhibited a home-base formation after 30 min observation time (Stewart et al., 2011). However, longer OFT recording time may be applicable to enhance the characterization of locomotor that lead to multiple complex behaviors in both Javanese medaka and zebrafish that remain to be discovered. Although top view for video recording provides an accurate tracking and quantification for both Javanese medaka and zebrafish behavior, further studies may utilize multiple cameras to generate three-dimensional behavior tracking. Moreover, manipulation of neuropharmacological agents will helps to increase our knowledge about home-base and locomotor phenotypes in both fish species. A previous study has shown that rodents demonstrated alteration in home-base specific locations when drug were given in different doses (Dvorkin et al., 2010). Therefore, the sensitivity of both fish species towards neuropharmacological treatment on their behavior in novel OFT merits further investigation.

Conclusion

Comparison of behavior between Javanese medaka and zebrafish in OFT tank provides fundamental insight into their locomotor, cognitive and affective phenotypes. Medaka exhibited an increase of exploratory activity with lower anxiety response in an open field-based paradigm and vice versa in zebrafish. Both fish species reaching a plateau in locomotion after mid phase demonstrating habituation responses were achieved. Javanese medaka exhibit a slow and steady habituation while zebrafish displayed a rapid habituation in the OFT tank. Both fish species exhibit similar pattern of thigmotaxis behavior. Both fish species displayed strong preference of using left eye to assess the OFT tank. Different fish species execute different locomotor behavioral phenotypes in an open field environment. The precision analysis of locomotion in fish models like Javanese medaka and zebrafish with high-throughput methods could provide accurate information needed for the screening and identification of neuroactive compounds. Collectively, this present study revealed that two different teleost aquatic model organisms, Javanese medaka and zebrafish have different behavioral phenotypes in open field test.

Abbreviations

OFT: Open field test

Declarations

Ethics approval and consent to participate

All experiments were performed in accordance with the rule of Institutional Animal Care and Use Committee of Universiti Putra Malaysia (UPM) (UPM/IACUC/AUP-R049/2019) and approved the procedures specific to this study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare there are no competing interests

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Authors’ contributions

NSAMS and NAB together conceived the idea, performed the laboratory work, analyzed and interpreted the data, and wrote the manuscript. NFR and NASH conducted behavioral recording procedures as single observer blinded to any treatment of the fish. WNWI supervised the project. All the authors have read and approved the manuscript.

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References

1. Amal, M.N.A., Ismail, A., Saad, M.Z., Md Yasin, I.S., Nasruddin, N.S., Mastor, S.S., Abdul Rahman M.H, Mohamad, N. (2019). Study on Streptococcus agalactiae infection in Javanese medaka (*Oryzias javanicus Bleeker*, 1854) model. *Microbial Pathogenesis*. 131:47-52. doi: 10.1016/j.micpath.2019.03.034.
2. Agnvall, B., Katajamaa, R., Altimiras, J., and Jensen, P. (2015). Is domestication driven by reduced fear of humans? Boldness, metabolism and serotonin levels in divergently selected red junglefowl (*Gallus gallus*). *Biology letters*, 11(9), 20150509. doi:10.1098/rsbl.2015.0509
3. Audira, G., Sampurna, B. P., Juniardi, S., Liang, S. T., Lai, Y. H., Han, L., & Hsiao, C. D. (2019). Establishing simple image-based methods and a cost-effective instrument for toxicity assessment on circadian rhythm dysregulation in fish. *Biology open*, 8(6), bio041871. https://doi.org/10.1242/bio.041871
4. Aziz, F.Z.A., Zulkifli, S.Z., Mohamat-Yusof, F., Amal, M.N.A. & Ismail, A. (2017).Histological study on mercury-induced gonadal impairment in Javanese medaka (*Oryzias javanicus). Turkish Journal of Fisheries and Aquatic Sciences*. 17 (2017), pp. 621-627.doi: 10.4194/1303-2712-v17_3_18
5. Baker, M. R., Goodman, A. C., Santo, J. B., and Wong, R. Y. (2018). Repeatability and reliability of exploratory behavior in proactive and reactive zebrafish, *Danio rerio. Scientific reports,* 8(1), 12114. doi:10.1038/s41598-018-30630-3
6. Bell, A. M., and Peeke, H. V. (2012). Individual variation in habituation: behaviour over time toward different stimuli in threespine sticklebacks (Gasterosteus aculeatus). *Behaviour*, 149(13-14), 1339–1365. doi:10.1163/1568539X-00003019
7. Belzung, C., and Philippot, P. (2007). Anxiety from a phylogenetic perspective: is there a qualitative difference between human and animal anxiety?. *Neural plasticity*, 2007, 59676. doi:10.1155/2007/59676
8. Bleeker, P. (1854). Ichthyologische waarneminsgen, gedaan op verschillende reizen in de Residentie Banten [Ichthyological observations made on different journeys in the Residence Banten]. Natuurkundig Tijdschrift voor Nederlandsch Indië, 7, 309-326
9. Berg, E. M., Björnfors, E. R., Pallucchi, I., Picton, L. D., & El Manira, A. (2018). Principles Governing Locomotion in Vertebrates: Lessons From Zebrafish. *Frontiers in neural circuits*, 12, 73. https://doi.org/10.3389/fncir.2018.00073
10. Champagne, D. L., Hoefnagels, C. C., de Kloet, R. E., & Richardson, M. K. (2010). Translating rodent behavioral repertoire to zebrafish (*Danio rerio*): relevance for stress research. Behavioural brain research, 214(2), 332–342. https://doi.org/10.1016/j.bbr.2010.06.001

11. Crispim Junior, C.F., Pederiva, C.N., Bose, R.C., Garcia, V.A., Lino-de-Oliveira, C., & Marino-Neto, J. (2012). ETHOWATCHER: validation of a tool for behavioral and video-tracking analysis in laboratory animals.*Computers in Biology and Medicine.* 42(2):257-64. doi: 10.1016/j.compbiomed.2011.12.002.
12. Dehmelt, F.A., von Daranyi, A., Leyden, C., &, Arrenberg, A.B.(2018). Evoking and tracking zebrafish eye movement in multiple larvae with ZebEyeTrack *Nature Protocol*. 13(7):1539-1568. doi: 10.1038/s41596-018-0002-0.
13. Dvorkin, A., Szechtman, H., & Golani, I. (2010). Knots: attractive places with high path tortuosity in mouse open field exploration. *PLoS computational biology*, 6(1), e1000638. doi:10.1371/journal.pcbi.1000638
14. Eilam, D. &, Golani, I. (1989). Home base behavior of rats (*Rattus norvegicus*) exploring a novel environment. *Behavioural Brain Research*. 34(3):199-211. doi.org/10.1016/S0166-4328(89)80102-0
15. Ferdaus, M.Y., Sarah-Nabila, A.G., Syaizwan, Z.Z., Amal, M.N.A., Wan-Norhamidah, W.I., Yusof, S. & Ismail, A. (2018) Acute toxicity test of copper pyrithione on Javanese medaka and the behavioural stress symptoms. *Marine. Pollution. Bulletin*., 127, pp. 150-153. doi: 10.1016/j.marpolbul.2017.11.046
16. Gaspary, K.V., Reolon, G.K., Gusso, D. and Bonan, C.D.(2018). Novel object recognition and object location tasks in zebrafish: Influence of habituation and NMDA receptor antagonism. *Neurobiology of Learning and Memory*, 155:249-260. doi: 10.1016/j.nlm.2018.08.005.
17. Godwin J., Sawyer S., Perrin F., Oxendine S.E., Kezios Z.D. (2012) Adapting the Open Field Test to Assess Anxiety-Related Behavior in Zebrafish. In: Kalueff A., Stewart A. (eds) *Zebrafish Protocols for Neurobehavioral Research. Neuromethods*, vol 66. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-61779-597-8_13
18. Gould, T.D., Dao, D.T., & Kovacsics C.E. (2009) The Open Field Test. In: Gould T. (eds) Mood and Anxiety Related Phenotypes in Mice. *Neuromethods*, vol 42. Humana Press, Totowa, NJ . https://doi.org/10.1007/978-1-60761-303-9_1
19. Gulyás. M., Bencsik, N., Pusztai, S., Liliom, H. and Schlett, K.(2019). AnimalTracker: An ImageJ-Based Tracking API to Create a Customized Behaviour Analyser Program. *Neuroinformatics*. 14(4):479-81. doi: 10.1007/s12021-016-9303-z.
20. Hall, C. S. (1934). Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. *Journal of Comparative Psychology*, 18(3), 385–403. doi:10.1037/h0071444
21. Higaki A, Mogi M, Iwanami J, Mins L-J, Bai H-Y, Shan B-S, et al. (2018) Recognition of early stage thigmotaxis in Morris water maze test with convolutional neural network. *PLoS ONE* 13(5): e0197003. https://doi. org/10.1371/ journal. pone.019700
22. Hong, X. and Zha, J.(2019). Fish behavior: A promising model for aquatic toxicology research. *The Science of Total Environment*, 686:311-321. doi: 10.1016/j.scitotenv.2019.06.028.

23. Horev, G., Benjamini, Y., Sakov, A., and Golani, I. (2007). Estimating wall guidance and attraction in mouse free locomotor behavior. *Genes Brain Behaviour*. 6, 30–41. doi: 10.1111/j.1601-183X.2006.00216.x
24. Hutter, S., Penn, D. J., Magee, S. and Zala, S. M. (2010). Reproductive behaviour of wild zebrafish (*Danio rerio*) in large tanks. *Behaviour*, 147, 641e660, doi:10.1163/000579510X12632972473944.
25. Huntingford F. A. (2004). Implications of domestication and rearing conditions for the behaviour of cultivated fishes. *Journal of Fish Biology*. 65, 122–142. 10.1111/j.0022-1112.2004.00562.x
26. Imai, S., Koyama, J, and Fujii, K.(2007). Effects of estrone on full life cycle of Java medaka (*Oryzias javanicus*), a new marine test fish. *Environmental Toxicology and Chemistry*. 26(4):726-31. doi: 10.1897/05-539r2.1
27. Inoue, K. and Takei, Y.(2002). Diverse adaptability in oryzias species to high environmental salinity.*Zoological Science*. 19(7):727-34. doi: 10.2108/zsj.19.727
28. Kalueff, A. V., Stewart, A. M., and Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. *Trends in pharmacological sciences*, 35(2), 63–75. doi:10.1016/j.tips.2013.12.002
29. Kiehn O., Dougherty K. (2016) *Locomotion: Circuits and Physiology*. In: Pfaff D., Volkow N. (eds) Neuroscience in the 21st Century. Springer, New York, N https://doi.org/10.1007/978-1-4939-3474-4_42
30. Kohda M, Hotta T, Takeyama T, Awata S, Tanaka H, Asai J-y, et al. (2019) If a fish can pass the mark test, what are the implications for consciousness and self-awareness testing in animals? *PLoS Biology.* 17(2): e3000021. https://doi.org/10.1371/journal.pbio.3000021
31. Koerber, A. S., & Kalishman, J. (2009). Preparing for a semiannual IACUC inspection of a satellite zebrafish (*Danio rerio*) facility. *Journal of the American Association for Laboratory Animal Science : JAALAS*, 48(1), 65–75. PMCID: PMC2694697
32. Kraeuter, A.K., Guest, P.C., and Sarnyai, Z.(2019).The Open Field Test for Measuring Locomotor Activity and Anxiety-Like Behavior. *Methods in Molecular Biology*, 1916:99-103. doi: 10.1007/978-1-4939-8994-2_9.
33. Kysil, E., Meshalkina, D., Frick, E., Echevarria, D., Rosemberg D et. al.(2017). Comparative Analyses of Zebrafish Anxiety-Like Behavior Using Conflict-Based Novelty Tests. *Zebrafish*, 14 (3) 197-208. doi: 10.1089/zeb.2016.1415
34. Leussis, M.P. and Bolivar, V.J.(2006). Habituation in rodents: a review of behavior, neurobiology, and genetics. *Neuroscience and Biobehavioural Reviews*. 30(7):1045-64. doi: 10.1016/j.neubiorev.2006.03.006
35. Lynn, D. A., & Brown, G. R. (2009). The ontogeny of exploratory behavior in male and female adolescent rats (*Rattus norvegicus*). *Developmental psychobiology*, 51(6), 513–520. https://doi.org/10.1002/dev.20386
36. Macrì, S., Neri, D., Ruberto, T., Mwaffo, V., Butail, S., and Porfiri, M. (2017). Three-dimensional scoring of zebrafish behavior unveils biological phenomena hidden by two-dimensional analyses. *Scientific*

reports, 7(1), 1962. doi:10.1038/s41598-017-01990-z

37. Matsunaga, W. and Watanabe, E.(2010). Habituation of medaka (Oryzias latipes) demonstrated by open-field testing. *Behavioural Processes*, 85(2):142-50. doi: 10.1016/j.beproc.2010.06.019.
38. Maurer, C.M., Huang, Y.Y., & Neuhauss, S.C. (2011).Application of zebrafish oculomotor behavior to model human disorders. *Review Neuroscience*. 22(1):5-16. doi: 10.1515/RNS.2011.003.
39. Mi, G., Gao, Y., Yan, H., Jin, X., Ye, E., Liu, S., Gong, Z., Yang, H. and Yang, Z. (2016). l-Scoulerine attenuates behavioral changes induced by methamphetaminse in zebrafish and mice. *Behavioural Brain Research*. 298(Pt A):97-104. doi: 10.1016/j.bbr.2015.09.039.
40. Miklósi, A., Andrew, R.J., and Savage, H.(1997). Behavioural lateralisation of the tetrapod type in the zebrafish (*Brachydanio rerio*). *Physiology and Behaviour*. 63(1):127-35. doi: 10.1016/s0031-9384(97)00418-6
41. Moretz J. A., Martins E. P., and Robinson B. D. (2007). Behavioral syndromes and the evolution of correlated behavior in zebrafish. *Behavioral Ecology*. 18, 556–562. 10.1093/beheco/arm011
42. Mustafa, A., Roman, E., and Winberg, S. (2019). Boldness in Male and Female Zebrafish (*Danio rerio*) Is Dependent on Strain and Test. *Frontiers in behavioral neuroscience*, 13, 248. doi:10.3389/fnbeh.2019.00248
43. Nema, S., Hasan, W., Bhargava, A. and Bhargava, Y.(2016). A novel method for automated tracking and quantification of adult zebrafish behaviour during anxiety. *Journal of Neuroscience Methods*, 271:65-75. doi: 10.1016/j.jneumeth.2016.07.004. Epub 2016 Jul 7.
44. Norton, W. H., Stumpenhorst, K., Faus-Kessler, T., Folchert, A., Rohner, N., Harris, M. P., … Bally-Cuif, L. (2011). Modulation of Fgfr1a signaling in zebrafish reveals a genetic basis for the aggression-boldness syndrome. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 31(39), 13796–13807. doi:10.1523/JNEUROSCI.2892-11.2011
45. Panula, P., Chen, Y. C., Priyadarshini, M., Kudo, H., Semenova, S., Sundvik, M., & Sallinen, V. (2010). The comparative neuroanatomy and neurochemistry of zebrafish CNS systems of relevance to human neuropsychiatric diseases. Neurobiology of disease, 40(1), 46–57. https://doi.org/10.1016/j.nbd.2010.05.010
46. Prut, L. and Belzung, C.(2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur. J. of Pharm. 463:3–33
47. Raderschall, C.A., Magrath, R.D., & Hemmi, J.M.(2011). Habituation under natural conditions: model predators are distinguished by approach direction. *The Journal of Experimental Biology*. 214(Pt 24):4209-16. doi: 10.1242/jeb.061614.
48. Rojas-Carvajal, M., Fornaguera, J., Mora-Gallegos, A. and Brenes, J. (Testing experience and environmental enrichment potentiated open-field habituation and groominsg behaviour in rats. *Animal Behaviour*, 137: 225-235. https://doi.org/10.1016/j.anbehav.2018.01.018
49. Rosemberg, D. B., Rico, E. P., Mussulini, B. H., Piato, A. L., Calcagnotto, M. E., Bonan, C. D., … de Oliveira, D. L. (2011). Differences in spatio-temporal behavior of zebrafish in the open tank paradigm

after a short-period confinement into dark and bright environments. *PloS one*, 6(5), e19397. doi:10.1371/journal.pone.0019397

50. Redish A. D. (2016). Vicarious trial and error. *Nature reviews. Neuroscience*, 17(3), 147–159. doi:10.1038/nrn.2015.30
51. Sakai, C., Ijaz, S., and Hoffman, E. J. (2018). Zebrafish Models of Neurodevelopmental Disorders: Past, Present, and Future. *Frontiers in molecular neuroscience*, 11, 294. doi:10.3389/fnmol.2018.00294
52. Saleem, S., and Kannan, R.R. (2018). Zebrafish: an emerging real-time model system to study Alzheimer's disease and neurospecific drug discovery. *Cell Death Discovery.* 4 (45). doi:10.1038/s41420-018-0109-7
53. Schnörr, S.J., Steenbergen, P.J., Richardson, M.K., and Champagne. D.L.(2012). Measuring thigmotaxis in larval zebrafish. *Behavioural Brain Research*. 228(2):367-74. doi: 10.1016/j.bbr.2011.12.016.
54. Seibeenhner, M. L., and Wooten, M. C. (2015). Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. *Journal of visualized experiments : JoVE*, (96), e52434. doi:10.3791/52434
55. Signore, I. A., Guerrero, N., Loosli, F., Colombo, A., Villalón, A., Wittbrodt, J., & Concha, M. L. (2009). Zebrafish and medaka: model organisms for a comparative developmental approach of brain asymmetry. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 364(1519), 991–1003. https://doi.org/10.1098/rstb.2008.0260
56. Sih A., Bell A. M., Johnson J. C., Ziemba R. E. (2004). Behavioral syndromes: an intergrative overiew. *The Quarterly review of biology.* 79, 241–277. Doi:10.1086/422893
57. Singer, M. L., Oreschak, K., Rhinehart, Z., and Robison, B. D. (2016). Anxiolytic effects of fluoxetine and nicotine exposure on exploratory behavior in zebrafish. *PeerJ*, 4, e2352. doi:10.7717/peerj.2352
58. Sovrano, V.A.(2004). Visual lateralization in response to familiar and unfamiliar stimuli in fish. *Behaviour Brain Research*. 152(2):385-91. doi: 10.1016/j.bbr.2003.10.022
59. Sovrano, V.A. and Andrew, R.J.(2006). Eye use during viewing a reflection: behavioural lateralisation in zebrafish larvae. *Behaviour Brain Research*. 167(2):226-31.doi: 10.1016/j.bbr.2005.09.021
60. Strykowski, J. L., & Schech, J. M. (2015). Effectiveness of recommended euthanasia methods in larval zebrafish (*Danio rerio). Journal of the American Association for Laboratory Animal Science : JAALAS*, 54(1), 81–84. PMCID: PMC4311746
61. Suriyampola, P.S., Shelton, D.S., Shukla, R., Roy, T., Bhat, A., and Martins, E.P. (2016). Zebrafish Social Behavior in the Wild. *Zebrafish,*13(1):1-8. doi: 10.1089/zeb.2015.1159.
62. Rankin, C.H., Abrams, T., Barry, R.J., Bhatnagar, S., Clayton, D.F., and Colombo, J. (2009).Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. *Neurobiology of Learning and Memory* 92(2):135–8.
63. Stewart, A.M., Gaikwad, S., Kyzar, E., and Kalueff, A.V.(2012). Understanding spatio-temporal strategies of adult zebrafish exploration in the open field test. *Brain Research.* 1451:44-52. doi:

10.1016/j.brainres.2012.02.064.

64. Thompson, S. M., Berkowitz, L. E., & Clark, B. J. (2018). Behavioral and Neural Subsystems of Rodent Exploration. *Learning and motivation*, 61, 3–15. doi:10.1016/j.lmot.2017.03.009
65. Yeh, C.M., Glöck, M., & Ryu, S. (2013). An Optimized Whole-Body Cortisol Quantification Method for Assessing Stress Levels in Larval Zebrafish. *PLoS ONE* 8(11): e79406. https://doi.org/10.1371/journal.pone.0079406
66. Yusof, S., Ismail, A., and Rahman, F.(2013). Distribution and localities of Java medaka fish (*Oryzias javanicus*) in Peninsular Malaysia. *Malayan Nature Journal*, 65(2and3), 38-46
67. Watkins, J., Miklósi, A., and Andrew, R.J.(2004). Early asymmetries in the behaviour of zebrafish larvae. *Behaviour Brain Research*.151(1-2):177-83. doi: 10.1016/j.bbr.2003.08.012
68. Wright D., Ward A. J., Croft D. P., Krause J. (2006). Social organization, grouping and domestication in fish. *Zebrafish* 3, 141–155. 10.1089/zeb.2006.3.14
69. Zabegalov, K.N., Kolesnikova, T.O., Khatsko, S.L., Volgin, A.D., Yakovlev, O.A., Amstislavskaya, T.G., Friend, A.J., Bao, W., Alekseeva, P.A., Lakstygal, A.M., Meshalkina, D.A., Demins, K.A., de Abreu, M.S., Rosemberg, D.B., and Kalueff, A.V. (2019).Understanding zebrafish aggressive behavior. *Behavioural Processes.* 158:200-210. doi: 10.1016/j.beproc.2018.11.010.
70. Zimmermann, A., Stauffacher, M., Langhans, W., and Würbel, H.(2001). Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. *Behavioural Brain Research*, 121(1-2):11-20. doi: 10.1016/s0166-4328(00)00377-

Figures

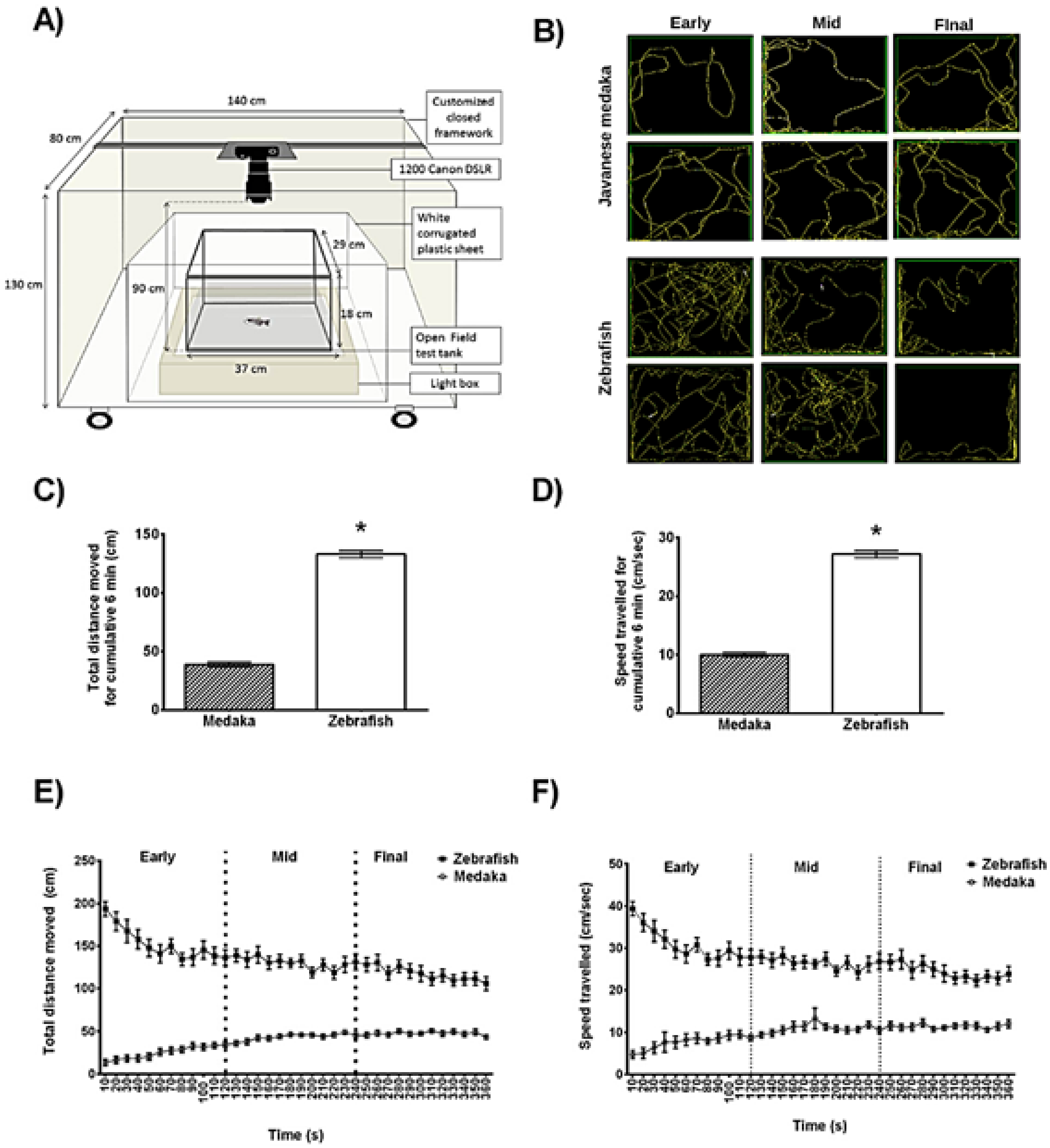

Figure 1

Locomotor behavior of adult Javanese medaka and zebrafish in a novel open field tank. (A) Schematic drawing of the behavioral setup for open field test behavioral assessment. (B) Swimming tracking of Javanese medaka and zebrafish during early, mid and final phase. (C-D) Total distance and speed travelled of Javanese medaka were slower compared to zebrafish over 6 minute trials. Decrement in locomotion represents reduction in exploration of a fish species. (E-F) Total distances and speed travelled in medaka were gradually increased during early phase and remain constants for the remaining of experimental period. In the other hand, total distances and speed travelled in zebrafish were decreased during remaining phase. Exploratory activity during early phase and habituation response of zebrafish in a novel arena during mid and final phase was higher than Javanese medaka. *Significantly different from Javanese medaka (P ≤ 0.05, n=30 for each fish species).

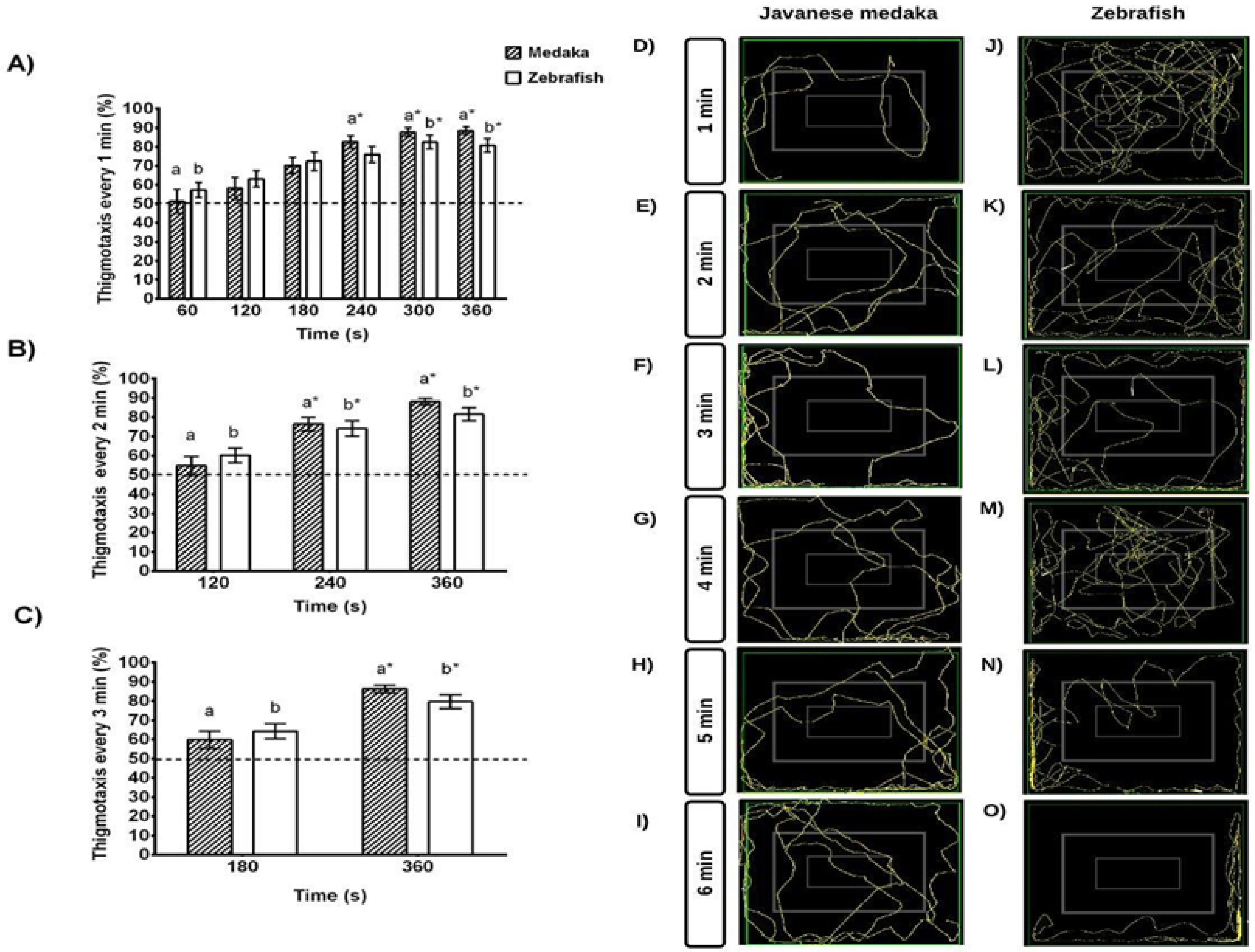

Figure 2

Optimization of data analysis for thigmotaxis behavioral evaluation. (A) Thigmotaxis analyzed for every 1 min intervals in Javanese medaka showed a significant increased at 240 s until 360s while thigmotaxis in zebrafish significantly increased at later time intervals, 300 s until 360 s. (B) Data analysis for 2 min intervals in both fish species showed a gradual increased in thigmotaxis. (C) Both fishes showed a prominent increment trend in thigmotaxis analyzed for 3 min intervals, suggesting this as the most ideal approach to analyze thigmotaxis behavior over 6 min trial. Thigmotaxis behavioral assessment during early phase may highly influenced by exploratory behavior. No significant difference in thigmotaxis between Javanese medaka and zebrafish over 6 min trials. (D-O) A prominent preference towards the edge of similar location over cumulative time may denote formation of home-based by Javanese medaka at 6 min while zebrafish started to form its home-based at earlier time, 5 and 6 min. The home-based formation were represented by the most intense yellow tracking at the edge of the OFT tank. * Significantly different from Javanese medaka ($P \leq 0.05$, n=30 for each fish species).

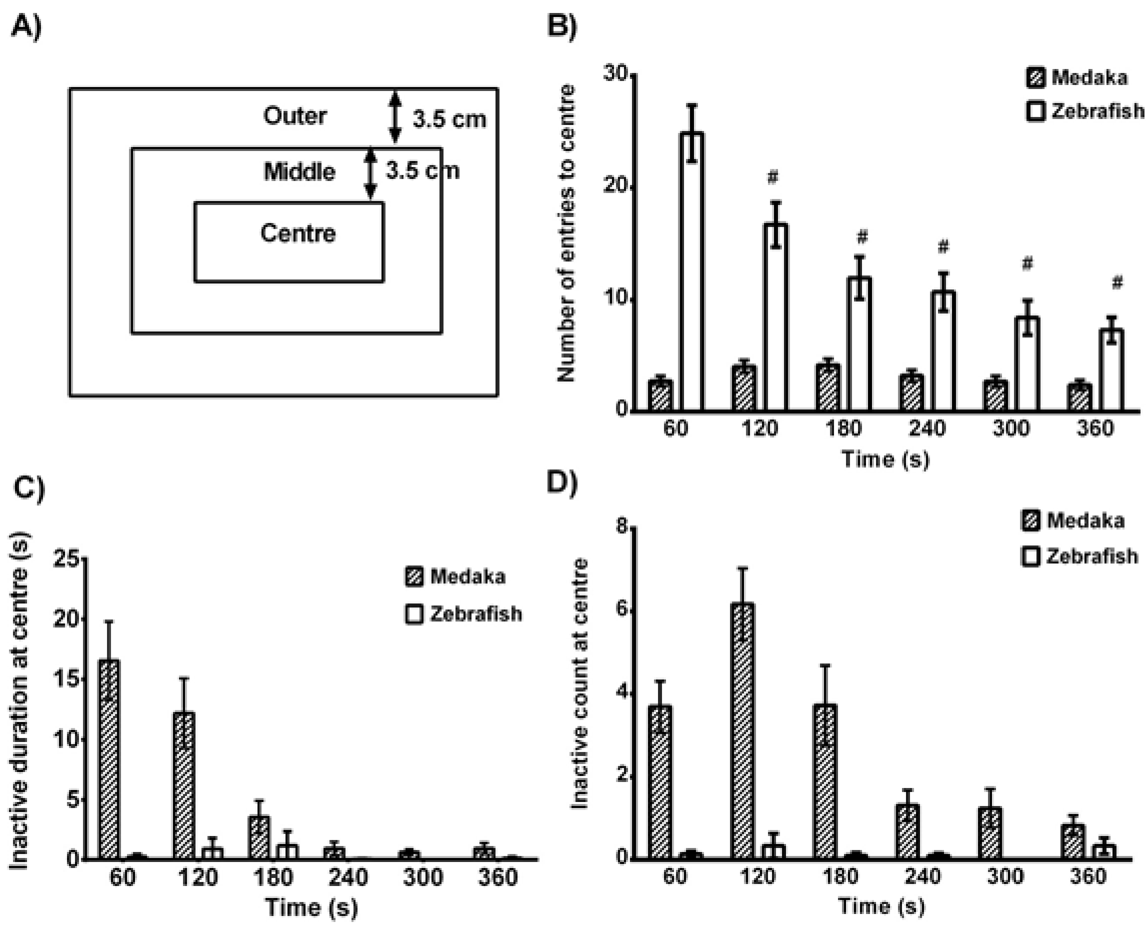

Figure 3

Comparison of activity profile according to different zone. (A) Schematic diagram of three main regions used in ZebraLab software, ViewPoint to analyze behavioral response in the OFT tank. (B) Zebrafish have higher number of entries to center region of OFT tank and decreasing over time in comparison to Javanese medaka which exhibit a fluctuation. These showed that both fishes may use the center and middle region as horizontal transitions to explore the OFT tank. (C) In the first 1 min, Javanese medaka remained inactive for more than 15 sec at the center showing that it steadily swum to the center in comparison to zebrafish which spent less than ~0.01 sec at the center and immediately swum toward the edge of OFT. (D) Medaka having higher inactive count in the center may represent they were less active or calmer than zebrafish. Since zebrafish achieved almost zero in inactive count at the centre and actively swimming to the other regions of OFT. *Significantly different from Javanese medaka ($P \leq 0.05$, n=30 for each fish species).

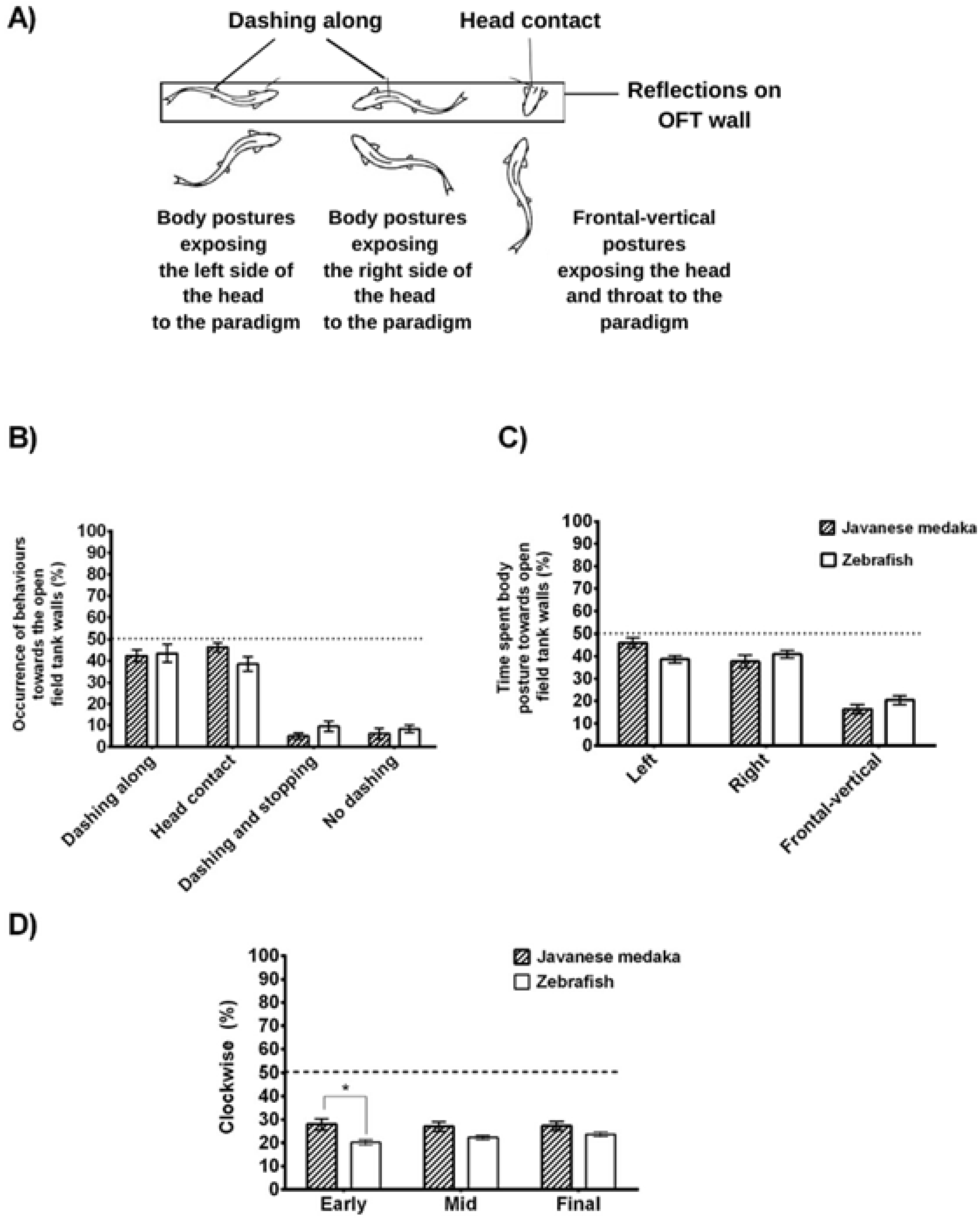

Figure 4

Other motor responses (A) Schematic diagrams for body postures orientation in OFT paradigm representing occurrence of other motor response: dashing along and head contact (B-C) No significant difference in occurrence of behaviors in a novel environment over 6 min and time body posture towards OFT wall between Javanese medaka and zebrafish. These showed that there were no significant difference of aggression between these two fish species (D) Both Javanese medaka and zebrafish displayed lower than 50% of clockwise body orientation throughout early, mid and final phase indicating preference of left eyes assessing a novel OFT paradigm.*Significantly different from Javanese medaka ($P \leq 0.05$, n=30 for each fish species).

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