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Computer Animation Technology in Behavioral Sciences: A Sequential, Automatic, and High-Throughput Approach to Quantifying Personality in Zebrafish (*Danio rerio*)

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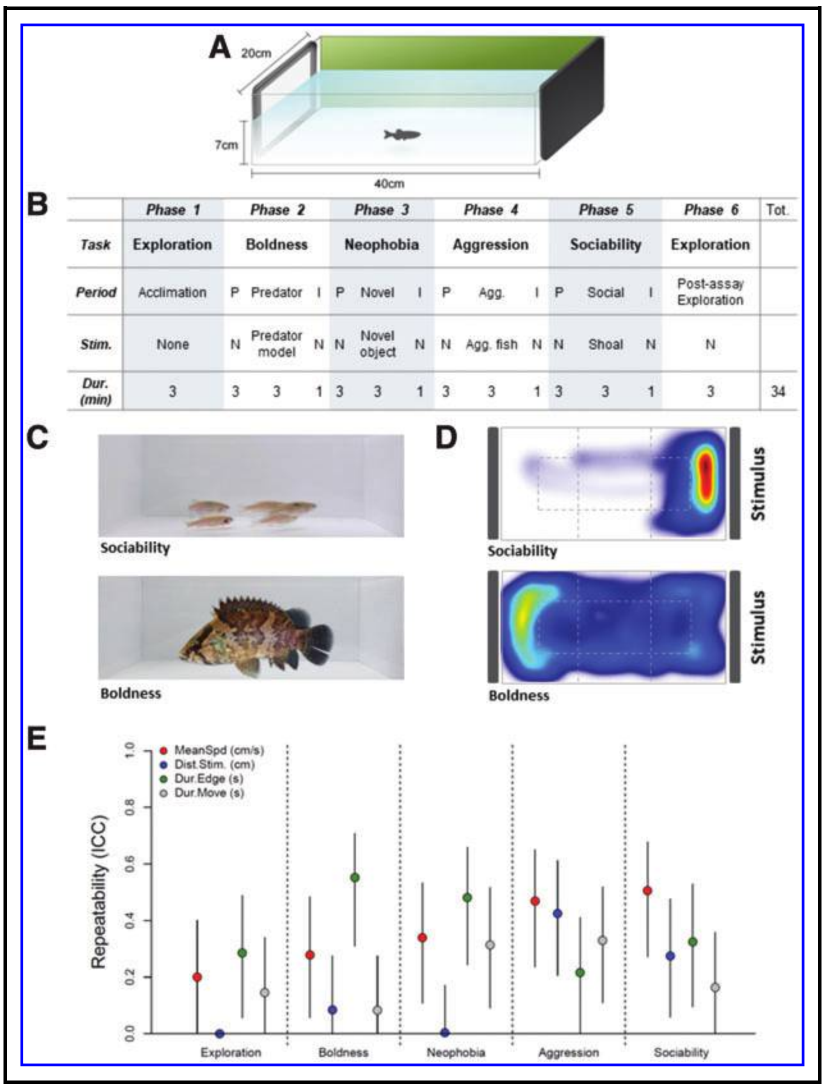

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Abstract

An emergent field of animal personality necessitates a method for repeated high-throughput quantification of behavioral traits across contexts. In this study, we have developed an automated video stimulus approach to sequentially present different contexts relevant to five ''personality'' traits (exploration, boldness, neophobia, aggression, and sociability), successfully quantifying repeatable trait measurements in multiple individuals simultaneously. Although our method is designed to quantify personality traits in zebrafish, our approach can accommodate the quantification of other behaviors, and could be customized for other species. All digital materials and detailed protocols are publicly available online for researchers to freely use and modify.

Keywords: behavioral phenotyping, automation, animal personality, consistency

Methods to rapidly quantify behavioral traits in model animal species are becoming increasingly important in the fields of neuroscience, behavioral sciences, and evolutionary biology.[1] Among behavioral traits, characterizing and quantifying ''personality'' traits are especially time-consuming due to the number of repeated measures that need to be taken to demonstrate individual trait repeatability (i.e., significant intraclass correlation, ICC[2,3]; for recent work on personality in zebrafish, see Ref.4). Fortunately, reduced costs and advances in computer technology permit not only repeated behavioral measurements of single traits but also the measurement of suites of behaviors, in a completely automated, standardized, customizable, and high-throughput manner without observer bias.[5] For example, tablet computers can present videos or animations to animals to elicit a behavioral response, while detailed behavioral measurements can be obtained with automated tracking software from video recordings. These types of methods have been explored previously in zebrafish,[1,6–13] but the application of multiple behavioral assays associated with personality in one continuous assay has not yet been implemented.

In this study, we have developed and trialed, using twenty-four adult zebrafish (male = 12; female = 12, approximately 6 months old), an automated video stimulus approach that sequentially presents behavioral contexts relevant to five personality traits: (1) exploration (movement in a novel environment), (2) boldness (response to a predator), (3) neophobia (response to a novel object), (4) aggression (response to a single aggressive conspecific), and (5) sociability (response to a shoal of conspecifics).

FIG. 1. **(A)** The experimental setup. Each tank had a *white* base to heighten subject contrast, and dark *green* walls. The nearest wall has been omitted for the visual representation of the inside of the tank. Two 8-inch computer tablets were placed against the transparent short walls of the tank, one blank (no stimulus, control) and the other displaying the stimulus video. **(B)** A timeline of tasks digitally presented to subjects, across a 34-min period. Stimulus videos were divided into six phases. Phases 1 and 6 are the exploration phases, where zebrafish are exposed to a neutral (N, no stimulus, control) background image. Phases 2–5 consist of four digitally simulated assays: boldness (exposure to an animated predator model), neophobia (exposure to an animated novel object), aggression (exposure to a video of a single aggressive zebrafish), and sociability (exposure to a video of a shoal of zebrafish). Phases 2–5 are pseudorandomized across trials, generating 24 unique trial combinations. **(C)** Representative frame from stimulus videos. The sociability assay consisted of a video of a zebrafish shoal (five fish), while the boldness assay consisted of an animated predator model swimming across the stimulus screen. **(D)** Heat maps generated by EthoVision for the sociability assay and boldness assay, demonstrating typical behavioral responses (distributions of fish location) that are elicited by these two stimuli. **(E)** Intraclass correlations (repeatabilities) and their 95% confidence intervals for four behavioral measurements (mean speed during movement in tank, mean distance of the fish to the stimulus, duration spent in the outer edge zone of tank, and duration moving in the tank) for the five behavioral assays associated with personality (exploration, boldness, neophobia, aggression, and sociability; see also Supplementary [See Supplementary Figures S1–S5; Supplementary Data are available online at www.liebertpub.com/zeb]). Color images available online at www.liebertpub.com/zeb

Behavioral trials occurred between 1 and 4 pm, over 4 days, at weekly intervals, measuring each individual four times over the course of the study (i.e., a subject was trialed once a week). For each trial, fish were placed individually into an experimental tank ($40 \times 12 \times 20$ cm, length $\times$ depth $\times$ width) filled to a water depth of 7 cm. Tanks were constructed from 6 mm acrylic consisting of a solid white floor, dark green long walls, and transparent short walls. On either side of the short walls, an electronic tablet (Windows 10 operating system, 8-inch screen, 1280×600 resolution) was positioned flat against the clear acrylic, projecting a computer-generated image to the experimental zebrafish (Fig. 1A). Thermostat-controlled heat mats (Ultimate Heat Mat, 40 W, 40×60 cm) maintained water temperature at $\sim 28°C$ for the duration of the trial.

Each trial lasted for 34 min, in which a single video consisting of six phases (Fig. 1B) was presented to the zebrafish. Phases 1 and 6, the ''exploration'' phases, consisted of a 3-min neutral background image (no stimulus, control). Phases 2–5, the ''stimulus'' phases, consisted of a 3-min prestimulus (no stimulus, control) period, a 3-min stimulus period, and a 1-min interval (no stimulus). Each stimulus phase comprised one of four tasks: social preference, novel object exposure, aggression, or predator avoidance (Fig. 1A, C). These tasks were designed to elicit a specific behavioral response (Fig. 1C and Supplementary Data; Supplementary Data are available online at www.liebertpub.com/zeb). Phases 2–5 were pseudorandomized, creating 24 possible video combinations. Trial videos were presented on either the left- or right-hand side of the tank, with the opposite tablet presenting a neutral (no stimulus) background image for the trial duration. Each zebrafish was randomly assigned to a tank and exposed to four unique videos and tanks, experiencing the stimulus on both the left- and right-hand sides of the tanks in a balanced manner.

Trials were filmed from above using a Panasonic HC-V770M camcorder (720p resolution). All videos were analyzed using EthoVision XT 11.5 automated tracking software. In this study, we present repeatabilities intraclass correlation, ICC,[3] for the four behavioral measurements (mean speed, mean distance to the stimulus, duration in the outer tank zone or edge, and duration moving) during the five personality assays (Fig. 1E). We found one or more significantly repeatable measurements, whose effect size was moderate to high, in each assay. These repeatable behavioral measurements can be used to compare individual or group differences, or look at across context correlations. Using the methods described herein (with the use of eight tanks), up to 64 individuals can be trialed within a 6-h period by one operator, allowing higher throughput designs to be implemented.

Notably, although we did find repeatable behavioral measurements under different contexts, it is premature to conclude that these five assays could completely capture five different personality traits.[14] Detailed analyses of different behavioral measurements under varied contexts are likely required to profile different animal personality traits. As somewhat expected,[15] our ''boldness'' and ''neophobia'' assays produced similar repeatability patterns in the behavioral measurements shown in Figure 1E, as did the ''aggression'' and ''sociability'' assays. However, in both cases, the degree of repeatability in the duration of moving could be a separating factor to distinguish different contexts (Fig. 1E). As pointed out before, however, we also recommend more than one assays to characterize a personality trait when possible.[16,17] In addition, different analysis profiles in EthoVision may allow for more detailed behaviors to be characterized, which better distinguish the different contexts (e.g., the number of zone transitions from the stimulus for characterizing aggressive behavior).

Importantly, all our stimulus videos, associated files, templates, and detailed protocols can be found online (Supplementary Data).[18] Therefore, researchers can use or customize our method with the use of available templates, depending on their specific experimental needs. For example, the 1-min interval we used between stimulus presentations can be easily prolonged to reduce a potential carryover effects, although such carryover effects can also be statistically dealt with.[19] Although we have designed our method using zebrafish, our approach can be easily redesigned for other similarly sized fishes, expanded to include more assays, and used with new, freely available, animal tracking software, for example, as in Ref.[20]

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Author Contributions

S.N., D.H., and D.N. conceived ideas and developed them with inputs from all the others. M.F., D.N., and S.N. designed the methodology. MF designed and generated all stimulus videos. M.F. performed the experiments with preparatory help from R.O., T.U., M.L., D.H., and D.N. D.N. analyzed the data. MF wrote the article with input from S.N. and D.N. M.F. wrote and collated all Supplementary Data with assistance from M.L., R.O., and D.N. All authors contributed critically to the drafts.

Disclosure Statement

The authors declare no conflict of interests.

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