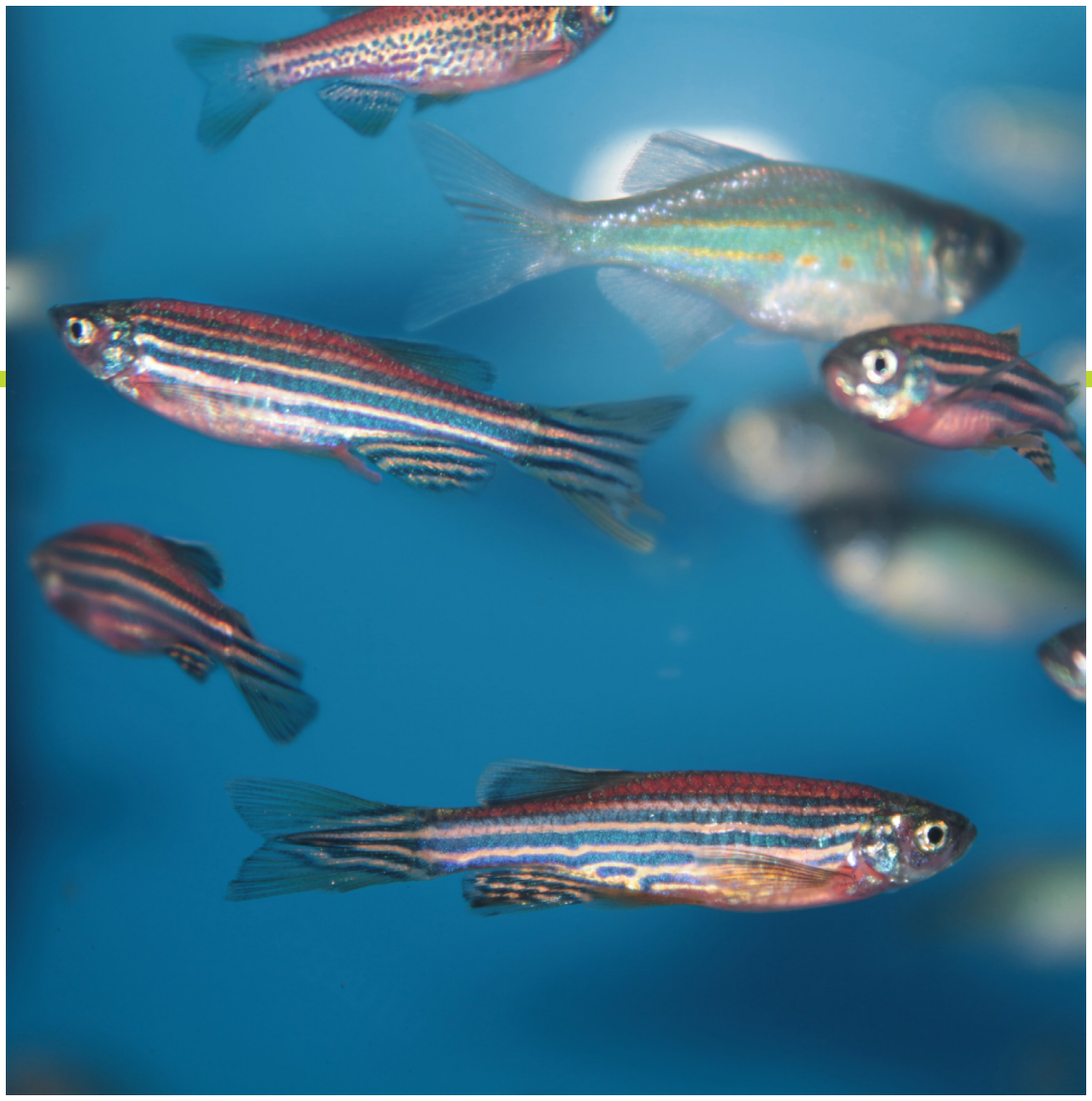


Novel tank diving test

How EthoVision XT can benefit your research

A white paper by Noldus Information Technology

NOVEL TANK DIVING, BOTTOM DWELLING

Exposed to a novel environment, zebrafish initially dive down to the bottom and then gradually start exploring.

The open field test is an established paradigm in rodent research, often used to measure exploration and anxiety-related behaviors. In zebrafish, this is no different [4,6,7]. Exposed to a novel environment, zebrafish initially dive down to the bottom and then gradually start exploring, first the bottom of the tank, then the top half [4]. This is typified as anxious behavior. Other typical behaviors that are associated with increased anxiety are inhibited exploration and speed of locomotion, and an increase in freezing behavior and erratic movements [4].

The use of video tracking is well-established in rodent studies on novel environments, and is now becoming more commonly used in zebrafish behavioral studies as well.

Video tracking with EthoVision XT is well-validated in research on rodents. Now more and more studies prove the value of EthoVision XT in zebrafish studies as well. Video tracking provides you with all the parameters that are interesting for the novel tank diving test – automatically and therefore objectively and efficiently.

METHODS AND MATERIALS

This paradigm, like the open field test in rodents, is often used for phenotyping [5], to test the effect of several drugs (anxiolytic or anxiogenic effects) [1,8,9,10,11,12], or effects of the environment (e.g. surface depth and substrate [2]).

Generally, wild-type (AB, short fin) zebrafish are used. Sometimes several species are compared to test the effect of genetics (e.g. leopard zebrafish [5]). Zebrafish are housed in groups, but tested individually.

Testing takes place in a novel tank, which can be a straight-forward aquarium and in most cases is atrapezoidal 1.5 liter tank. This tank is visually divided into two [2,5,4,8,9,11,12,4] or three [1,10] horizontal zones to objectively determine bottom dwelling or top swimming behavior.

EthoVision XT has intuitive drawing tools that let you easily define the zones of interest in the video image of your aquarium. This way you can define horizontal zones (top, bottom, and center), or any other zones you are interested in. Behavior measured during the experiment is automatically linked to these zones.

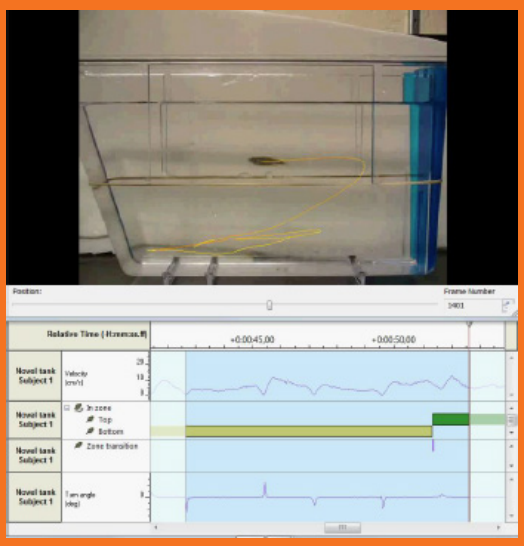
USE OF PARADIGM

A substantial part of the experiments are aimed at testing the effect of certain drugs.

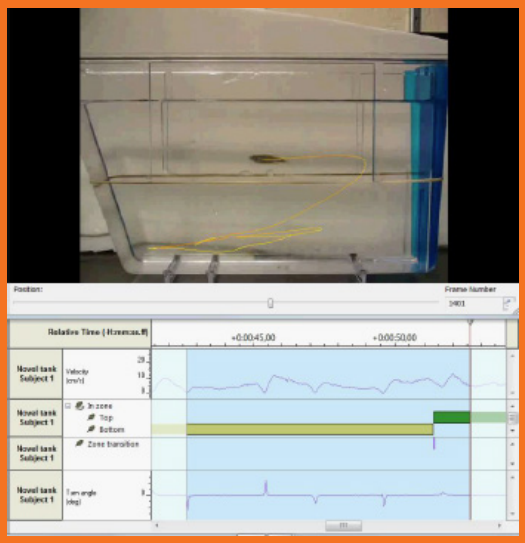
The novel tank diving test can be used to investigate different effects of the new environment on fish behavior. For example, Bencan *et al.* tested the effect of the size of the home cage on novel tank behavior [1]. Blaser and Goldsteinholm [2] tested the effect of different surface depths and substrates, while Champagne *et al.* [7] investigated the effect of the restraint test.

A substantial part of the experiments are aimed at testing the effect of certain drugs, mostly anxiogenics or anxiolytics, such as Buspirone, Chlordiazepoxide, and Diazepam [1,10], Fluoxetine, Ethanol, and Morphine [5], Caffeine and LSD [8], Mescaline and PCP [9], Nicotine [10], or RDX [12].

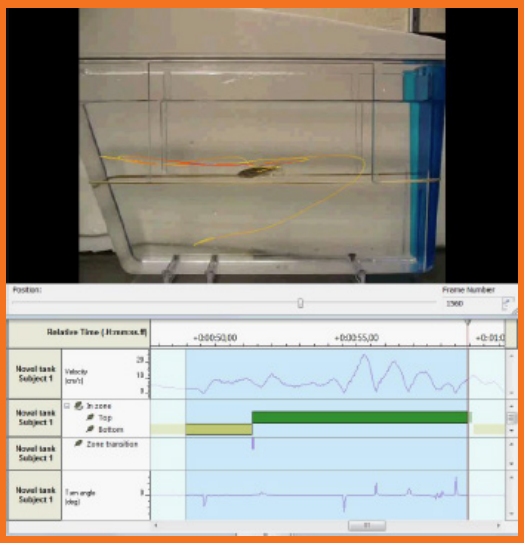
In the novel tank diving test, the zebrafish typically first dives down to the bottom. It might freeze there for a while, and then it starts exploring, first the bottom of the tank, then the more upper part.



EthoVision XT automatically tracks the movement of the zebrafish and accurately determines the (first) crossing to the upper part. You can see this in the track and registered in the two middle graphs.



Distance swum and swim speed are also measured automatically. Coloring in the tracks as well as the top graph shows variation in speed.

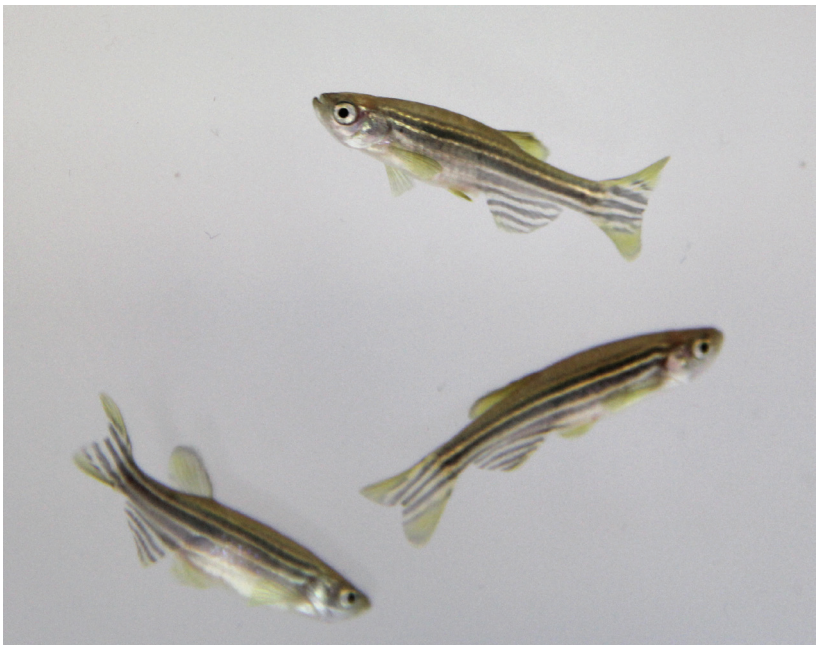


Turn angle represents the animal turning. In this picture, the animal just turned around as you can see in the track and the lower graph.

PARAMETERS

There are several parameters that are commonly used in the novel tank diving test. These are:

- The **latency to top zone** reflects how long it takes the fish to enter the top zone for the first time (in seconds) is often of prime interest because it reflects anxiety-like behavior [5, 8, 9, 11].
- The **time spent in zone**, bottom, top, or center, is often expressed in seconds or in percentage of total test time [1,2,4, 7,8,9, 10, 11,12].
- The **distance from bottom** - the mean distance from the bottom during the test - is another parameter that is sometimes used. [2]
- The **number of transitions** reflects the amount of crossings between the zones and is often expressed as total number or amount per minute [5,7, 9, 11, 12].
- **Velocity**, often expressed as mean and maximum speed of swimming, reflects activity of the fish [1, 4, 7, 8, 11].
- The **distance swum** or **total path length** also reflects activity [1,2,4, 7, 8,10, 11].



- **Mobility**, sometimes called **activity,** says something about how much time the zebrafish spent swimming or immobile (frozen). This can be expressed in seconds or in relative time [1, 2,5, 4, 7, 8,9, 11, 12].
- **Erratic movements** are the typical sharp changes in swimming direction shown by zebrafish [5, 4, 11, 12].

EthoVision XT automatically tracks and analyzes the above mentioned parameters. Erratic movement, for example, can be tracked automatically.

EthoVision XT has a built-in manual event recorder (MER), an ideal tool to score behaviors manually simultaneous to the automatic tracking. It is a practical tool to score detailed behavior, or you can use it to validate and fine-tune parameters that are automatically calculated, such as mobility.

WHAT TO EXPECT - RESULTS FROM SCIENTIFIC RESEARCH

INFLUENCE OF ANXIOLYTIC AND ANXIOGENIC MANIPULATIONS

Anxiolytic drugs or other anxiety-reducing manipulations decrease the latency to explore the top part of the aquarium. They cause the fish show less erratic movements, less and shorter freezing bouts, and they spend more time in the top part and less in the bottom. Bencan *et al.* [1] found that buspirone and diazepam had these anxiolytic effects, while chlordiazepoxide did not. Levin [10] also notes this significant anxiolytic effect of buspirone and diazepam, and found that the more atypical nicotine produced this effect as well, while, again, chlordiazepoxide did not.

Cachet *et al.* [5] confirmed the anxiolytic effects of fluoxetine, ethanol, morphine, and nicotine. They also confirmed several anxiogenic conditions: acute pre-exposure to alarm pheromone and repeated morphine withdrawal both resulted in significant results for all behavioral parameters. Using a leopard strain and pre-exposure to caffeine also showed significant anxiogenic effects for at least half of the parameters tested.

EFFECTS OF PSYCHEDELIC DRUGS

Grossman *et al.* [8] tested the effects of LSD and found that it had significant anxiolytic effects. Control subjects showed normal behavior: they initially dove to the bottom, froze, and then gradually increased their activity and started exploring the top. Normally, fish first explore in horizontal dimensions, then in vertical. LSD caused fish to swim in both dimensions with high amplitude, they visited the top half sooner, longer, and more often and showed less freezing.

Kyzar *et al.* [9] found similar effects of mescaline and PCP. They found more highly mobile phases, while periods of immobility were shorter in duration.

INFLUENCE OF THE HOME TANK AND TESTING ENVIRONMENT

When zebrafish are tested in a tank that has different dimensions than their home tank, this influences their behavior. Bencan *et al.* [1] describe this in their paper. Fish that are tested in a smaller tank (1.5L) than their home tank (3L) showed a lower swim speed, more diving response in the beginning of

the test, and less later on in the test compared to a group that had the same size home and test tank.

Champagne *et al.* [7] found that zebrafish show hyperactivity during initial exposure to a novel environment and that acute stress momentarily greatly attenuated thigmotaxis.

INFLUENCE OF SURFACE DEPTH AND SUBSTRATE

Blaser and Goldsteinholm [2] created an interesting apparatus with a split depth tank. The substrate floor (grey gravel) was separated from the accessible part of the aquarium by a glass plate. This glass plate could be moved up or down, so that it was close to or further away from this gravel floor. This way, both surface depth and substrate depth could be adjusted. The authors found a clear preference for greater surface depth in wild type zebrafish. Interestingly, when there was no difference in surface depth, the zebrafish seemed to prefer the part of the aquarium with more substrate depth than the part with less substrate depth.

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