

## Supporting evidence and references

### Zebrafish behaviour and welfare posters

#### Zebrafish enrichment: Natural environment

- Wild zebrafish are found in habitats with a variety of vegetation, substrates and water flow rates<sup>1-4</sup>.

#### Zebrafish enrichment: Plants

- Including plants in the tank is preferable to leaving it barren<sup>5,6</sup>.
- Environments containing artificial plants may contribute to lower anxiety<sup>7,8</sup>, reduced stress response<sup>9</sup>, social cohesion<sup>10</sup>, better learning and memory<sup>11,12</sup>, larger brain size<sup>7</sup>, and improved fertility and fecundity<sup>13</sup> for zebrafish. They also prevent oxidative stress<sup>9</sup> and improve the fish's ability to cope with pain<sup>8</sup>.
- We recommend monitoring social behaviour after adding plants to the tank, to see if the zebrafish's behaviour changes<sup>14-17</sup>.

#### Zebrafish enrichment: Substrate

- Using gravel, sand – or even an image of gravel – in a tank is preferable to a barren environment<sup>5</sup>.
- Environments containing substrate may contribute to lower anxiety<sup>18</sup>, reduced stress response<sup>9,19</sup>, larger brain size<sup>7</sup>, and higher rates of improved larvae survival<sup>18</sup> for zebrafish. They also prevent oxidative stress<sup>20</sup> and improve the fish's ability to cope with pain<sup>8</sup>.

#### Zebrafish enrichment: Shelter and structure

- Tanks that contain structure such as rocks and shelters are preferable to barren tanks<sup>5,6</sup>.
- Structured environments may contribute to lower anxiety, reduced stress response, increased exploration, improved learning, and larger brain size in zebrafish, and improve their ability to cope with pain<sup>7-9,19</sup>.

#### Zebrafish enrichment: Live food

- Feeding zebrafish live food is widely used to promote short-term enrichment by stimulating their natural predatory behaviour<sup>21</sup>.
- Organisms fed to zebrafish, such as rotifers or *Artemia nauplii*, are not considered sentient.

#### Zebrafish enrichment: Group housing

- It's preferable to keep zebrafish in group housing rather than on their own or in pairs<sup>22-24</sup>, as group housing contributes to lower anxiety and faster recovery from stress<sup>22,25,26</sup>.
- More information is needed on preferred group sizes, however.
- Aggression may be high in small groups of zebrafish (fewer than six fish), so caution should be taken and the fish carefully monitored<sup>27</sup>.
- Where fish need to be housed alone or in pairs, using clear, perforated barriers will maintain visual and olfactory contact. Providing other enrichment such as plants can help reduce stress<sup>19,22,28</sup>.

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## Zebrafish enrichment posters



Scan for the Natural Environment poster.



Scan for the Group Housing poster.



Scan for the Live Food poster.



Scan for the Plants poster.



Scan for the Shelter and Structure poster.



Scan for the Substrate poster.

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