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Maturation of behaviour in the fathead minnow

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ABSTRACT

Fathead minnow (FM, *Pimephales promelas*) are a species of small fish native to North America. Their small size, fast development, and ability to breed in the lab make them an ideal species to use in research, especially in toxicology. Behaviour in general is poorly studied in FM. The aim of this study was to characterize the normal behaviour of fathead minnow at 3 different stages of development in a light-dark box and in a social behaviour test. Fish larvae showed a preference for the light area, and then an increase in dark preference was seen as the fish aged. FM preferred to be with conspecifics at each age, but this preference was much stronger at the adult stage. The time of first entry into the conspecific area was reduced with increasing age of the fish. The time spent in the conspecific area increased between the juvenile and adult stage, and adults stayed more in this area when they entered it. Maturation of behavior in FM was demonstrated in our study. The FM is another good model fish to assess behavioral effects of chemicals, and this study helps to define the appropriate ages for behavioral studies with FM.

1. Introduction

Fathead minnows are small fishes native to North America. Their small size, fast development and ability to breed in the lab makes them an ideal species to use in research, especially in toxicology (Colavecchia et al., 2004; Ankley and Villeneuve, 2006; Floyd et al., 2008).

Behaviour in general is poorly studied in Fathead minnows. Some publications have investigated reproductive behaviour. Cole and Smith (1987) studied male courting behaviour in fathead minnows. Later studies assessed FM reproductive behavioural parameters to study the impacts of salinity (Hoover et al., 2013) or pollutants (Schoenfuss et al., 2008; Wang et al., 2014). Avoidance behaviour with fluoranthene (Farr et al., 1995) and predator avoidance (McGee et al., 2009) have been investigated as well.

The aim of this study was to characterize the normal behaviour of fathead minnow at 3 different stages of development: larvae, juveniles and adults. Two different kind of behaviour were investigated. The first one is the light-dark box test which evaluates the preference for dark or light in fathead minnows. The second test investigates the social behaviour, assessing whether the fish prefers to be with cohorts or alone. Fathead minnow are endemic fish from North America and we chose to use this species (instead of zebrafish) as it is more environmentally- relevant in an ecotoxicological context to the North-American environment.

Previously the light-dark box has been used with zebrafish (*Danio rerio*) (Serra et al., 1999; Gerlai et al., 2000; Stephenson et al., 2011) to determine preference. The light-dark box has also been used to test conditioned choice place preference in sea bass (*Dicentrarchus labrax*) (Millot et al., 2014) and in zebrafish (Ninkovic and Bally-Cuif, 2006; Kedikian et al., 2013). Application of different background colors was recently investigated. For example, Gaffney et al. (2016), analyzed background color effects on aggressiveness in Coho salmon (*Oncorhynchus kisutch*) in aquaculture. In the current study, this test was used to determine light-dark preference in fathead minnows at different ages with the aim of developing the test for use in an ecotoxicological context later on to determine if exposure to toxicants can affect light-dark preference behaviour.

Fathead minnows are a socialising fish. They live groups like other fish (such as zebrafish). Social preferences were investigated with zebrafish adults without exposure (Blaser and Gerlai, 2006; Saverino and Gerlai, 2008; Pham et al., 2012) or with exposure to compounds such as LSD (Grossman et al., 2010) or alcohol (Gerlai et al., 2000), or herbicides such as paraquat (Bortolotto et al., 2014). Social preferences in guppies exposed to oil (Jacquin et al., 2016) and social differences between zebrafish and black neon (*Hyphessobrycon herbertaxelrodi*) (Gimeno et al., 2016) were also investigated. This test has also been used to analyse disruption of social preference (Scerbina et al., 2012). Social studies were conducted in mosquitofish (*Gambusia affinis*) (Polverino et al., 2013) and used to understand the impacts of domestication on sea bass (*Dicen-*

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trarchus labrax)(Benhaïm et al., 2013). Pollutant effects on social behaviours were also investigated in Gilthead seabream (*Sparus aurata*) juveniles, where phenanthrene exposure decreased their social interaction (Correia et al., 2007) and with zebrafish exposed to various drugs (Schaefer et al., 2015). The goal of these experiments was to assess control FM behaviours using the light-dark preference and social interaction tests. Later on, the two tests were adapted for use in an ecotoxicological context, after exposure of FM to various toxicants, to identify some behavioural changes that occurred after exposure. These behavioural changes (such as predator avoidance, environmental sensory awareness, or shoaling cohesion) can be very important for fish survival. So the behavioural endpoints can be useful additions to toxicological assessments which traditionally focus on survival, growth, and reproduction.

To our knowledge, there have been no publications related the normal behaviour of fathead minnow in light-dark box test or for social preference. The hypothesis was that fathead minnow's behaviour and preferences will become stronger with age, as occurs in zebrafish (Buske and Gerlai, 2012; Mahabir et al., 2013).

2. Methods

2.1. Fish

All the eggs were purchased from Aquatox (Guelph, ON) and raised in the laboratory in Burlington, ON. They were raised from eggs until 16 dph in beakers in a 25 °C incubator and then transferred into flow-through aquaria until adulthood. They were fed twice a day with fresh *Artemia* nauplii between 1 and 65 dph and with frozen brine shrimp also given at the juvenile stage (after 30 dph).

2.2. Behavioural experiments

All experiments were approved by the animal care committee at Environment Canada, under Animal Use Protocol 1411, Standard Operating Procedure for Fathead Minnow Lifecycle Assays, and Standard Operating Procedure for Fathead Minnow Behavioural Assays.

All behavioural tests were performed in a dedicated Environmental chamber with controlled temperature and light parameters. Fish were minimally stressed by assuring that the room was kept at the same conditions as in exposure aquaria with respect to temperature and lighting. Temperature was 27 °C and the light was 450 Lux. Fish were acclimated in groups of 5 one hour before the test in a holding tank. Thirty fish were tested at the larval stage (16 days post-hatch, dph), 60 fish at the juvenile stage (60 dph), and 60 fish at the adult stage (30 males and 30 females at 140 dph). The light-dark box test was performed in the morning. Fish were acclimatized for 1 h again and tested in the afternoon for social preference. All videos were analyzed manually by the same person.

2.3. Light-dark box test

The aim of the test is to assess whether the fish prefer to be in light or dark surroundings, and if this preference increases or changes with age. This test was adapted from Cachat (2013). Two configurations of the light-dark box aquarium were built from Plexiglas: one for 16 dph larval fish and one larger for 60 dph juvenile and 140 dph adult fish. The measurements for the smaller light-dark box (larval fish) were 15 cm in length, 10 cm tall, and 10 cm deep (1.5 L volume) and the measurements for testing larger fish were 30 cm in length, 20 cm tall, and 20 cm deep (12 L volume). Half of each box was covered by opaque black plastic (walls, floor and lid).

After the 1 h acclimation, individual fish were placed in the light area and the recording video started. Behaviour was recorded for 6 min. At the end of this time, fish were returned to their holding aquaria in the environmental chamber after each assessment.

Observations recorded were the total time (in sec) spent in the dark and in the light area, the time of the first entry into the dark area (in sec), the number of entries into the dark area (total and during the first minute), the mean time (in sec) spent in the dark area (percentage), and the place of the fish at the end of the test.

2.4. Social preference test

The aim of this test is to assess whether the fish prefers to be with other fish, or whether it prefers to be alone. This test is adapted from Cachat (2013). One size of test chambers was created for larvae and one for juvenile and adults. The test box is composed of 3 boxes (two external small boxes and one big box in the centre). The two external boxes are a large area 7 cm in length, 10 cm tall and 10 cm deep (0.7 L volume) for larvae or 15 cm in length, 20 cm tall, and 20 cm deep (6 L volume) for juveniles and adults. They are physically separated from the central main box by a transparent wall. These two boxes are used to hold the conspecific fish (conspecific box) or no fish (empty box). The central main box is equivalent to the 3 small ones, virtually separated in 3 parts: one is the empty part (near the empty box), one is the middle part, and the third is the conspecific part (near the conspecific box). Measurements were 21 cm in length, 10 cm tall and 10 cm deep with a volume of 2.1 L for larvae and 45 cm length, 20 cm tall and 20 cm deep with 18 L of volume for juveniles and adults. To avoid lateral bias in the experimental cohorts, the left/right location of the conspecific box with fish was alternated between trials.

Individual fish were placed in the middle area of the centre box and confined in this place with transparent walls for 30 s. After this time, the walls were removed and the fish could freely swim in all of the central area (near conspecific, middle or near the empty areas). Fish were observed for 6 min in the centre test area. At the end of this experiment, fish were returned to acclimatization (holding) aquaria after each assessment. At the end of the day they were returned to their exposure aquaria and were fed.

Time in each area (conspecific part, middle part and empty part) (sec), first entry into conspecific part (sec) and into empty part (sec), number of entries into conspecific part and empty part, mean time spent in conspecific part and empty part, and place of the fish at the end of the test were recorded.

Conspecifics were composed of 5 fish of the same age (Gerlai et al., 2000) from another tank. They were acclimated 1 h before the social preference test. Five immature fish were randomly chosen at 16 and 60 dph. At 140 dph, 5 mature females were placed in the conspecific box when males were tested and 5 mature males were randomly placed in the conspecific box when females were tested.

2.5. Statistical analysis

Statistical analyses were carried out with Statistica 9.0 (Statsoft, Tulsa, OK, USA) software. Generalized linear models (GLM) were applied in each case, for statistical assessment of the effect of age on measurements of light-dark box test (LD) and social preference. Tukey's HSD was used as posthoc test. In LD, we tested age effect (16, 60 or 140 dph) as fixed factor for all tests. Area effects (dark or light) as fixed factors as well and interaction between age and areas were tested only for the endpoint "time in each area" and "place at the end". For the other test, only the results in the dark area were taken into account. Sex (immature, male, or female) and fish (number of fish tested) effects were tested in each analysis as random factors. In the results section, only significant fixed factor or interaction effects were reported to simplify the text, but all the results are reported in Sup Table 1. No sex effects were observed in differences between males and females at 140 dph for the LD. All the 140 dph fish (males and females) were pooled for this test.

In the social preference test, age effect, area effect, and interaction between age and area were tested as fixed factors for all tests. Sex (immature, male, or female) and fish (number of fish tested) effects were tested in each analysis as random factors.

No sex effect was observed for social behaviour, except for the number of entries into each area. In this case only, another GLM was applied to investigate differences between males and females at 140 dph. Sex and area were tested as fixed factors and fish number as a random factor. The interaction between sex and area was tested as well for "the number of entries into each area" in the social preference test.

3. Results

3.1. Light-dark box test

3.1.1. Time in areas

We assessed the light dark box test to see any preference in dark or light with age at the larval (16 dph), juvenile (60 dph) and adult (140 dph) stages. Results (Fig. 11 and Sup Table 1) showed an area effect ($F = 49.64$; $p < 0.0001$, $n^2{}_p = 0.18$) and an interaction between age and area ($F = 35.04$; $p < 0.0001$, $n^2{}_p = 0.23$). Time spent in the dark area increased with age (140 dph >60 dph, with $p = 0.006$ and 60 dph >16 dph with $p = 0.039$). Larval fish spent on average 156 s (s) in the dark area while juvenile fish stayed in this area around 227 s and 295 s for adults. At the same time, time in the light area decreased from 203 s on average at larval stage, to 133 s at the juvenile stage, and 65 s at the adult stage (140 dph <60 dph, with $p = 0.006$ and 60 dph <16 dph with $p = 0.039$).

3.1.2. Time of first entry into dark area

Fish were placed in the light part of the box at the beginning of the experiment and the first entry in the dark part was monitored (Fig. 12 and Sup Table 1). An age effect was found ($F = 10.03$; $p = 0.002$, $n^2{}_p = 0.10$) with a decrease in the time of dark first entry between 16 and 60 dph ($p = 0.002$). Time for dark first entry decreased from an average of 48 s at 16 dph to 12 s at 60 dph, and to 6 s at 140 dph. At 140 dph, latency was different from 16 dph ($p = 0.0004$) but not from 60 dph ($p = 0.767$).

3.1.3. Number of entries into dark area

In this part, total number of entries (Fig. 13 and Sup Table 1) and entries in the first minute (Fig. 14 and Sup Table 1) were analyzed. This gave an idea of the amount that the fish were moving between light and dark sides. There were differences in the total number of entries with fish age ($F = 4.79$, $p = 0.031$, $n^2{}_p = 0.05$) but it was not possible to track it to the level of the factors.

An age effect was found for number of entries in the first minute ($F = 7.65$; $p = 0.007$, $n^2{}_p = 0.08$). Post hoc test revealed that 16 dph was different from 60 and 140 dph ($p = 0.004$ and $p = 0.009$ respectively). In the first minute fish had 1.5 entries on average at 16 dph compared to 3 and 2.9 entries respectively, at 60 and 140 dph.

3.1.4. Mean time spent in dark area

Mean time spent in the dark area was calculated as the total time spent in dark area divided by the number of entries into the dark part. No significant effects were observed in this test at $p = 0.05$ but an age effect was seen with $p = 0.1$ ($F = 3.34$, $p = 0.071$, $n^2{}_p = 0.04$). In this case, we observed similar results in larvae and juveniles ($p = 0.320$) and (Fig. 15 and Sup Table 1) There was a significant increase in mean time in the dark area between the juvenile and adult stages ($p = 0.0002$) from 57 to 150 s.

3.1.5. Place at the end

The last variable is the place of the fish at the end of the test. At each age the percentage of fish in the dark part at the end of the test was recorded (Fig. 16 and Sup Table 1). No age difference was found with statistics ($F < 1$; $p = 1.0$, $n^2{}_p = 0.00$) but an area effect ($F = 27.35$, $p < 0.0001$, $n^2{}_p = 0.10$) and an interaction effect between age and area ($F = 8.94$, $p < 0.001$, $n^2{}_p = 0.07$) were found. A tendency to finish in the dark part increase with the age of fish and was significant in adults ($p < 0.0001$). At the end of the test 53% of larvae were in the dark box, while 62% of juveniles and 83% of adults ended the 6 min test in the dark area.

3.2. Social preference test

3.2.1. Time in areas

Distribution of time between the 3 areas looked similar for fish of all ages (Fig. 21 and Sup Table 1). Fish preferred the conspecific part of the box at all ages (258 s at 16 dph, 220 s at 60 dph and 325 s at 140 dph). The GLM confirmed an area effect ($F = 223.75$; $p < 0.0001$, $n^2{}_p = 0.54$) where each area was different from the other ($p < 0.001$ in each case) and an interaction between age and area ($F = 14.19$; $p < 0.0001$, $n^2{}_p = 0.13$). At 16 dph, 60 and 140 dph, the time spent in conspecific area was different from time in middle and empty ($p < 0.0001$ for all of them). There were no differences between these two other boxes except at 60 dph ($p = 0.002$) were time in empty box is more important than time in middle box. Juveniles clearly showed a preference for the conspecific area (220 s), then for the empty area (107 s) and for the middle (34 s; $p \leq 0.001$ for all). The comparison of time spent at each age in the conspecific area revealed an increase in time in the conspecific area for adults in comparison to juveniles ($p < 0.0001$). At the same time, a decrease of time in the empty box was observed in the adult fish (with 16 dph (74 s), 60 dph (107 s),140 dph (20 s) with $p < 0.0001$ between adults and juveniles).

3.2.2. First entry into each area

Fish were placed in the middle area at the beginning of the test. After 30 s, the transparent walls were removed and the time of first entry into the conspecific area or the empty area was recorded (Fig. 22 and Sup Table 1). There was an area effect ($F = 24.84$; $p < 0.0001$, $n^2{}_p = 0.09$) and an interaction between area and age ($F = 4.23$; $p = 0.016$, $n^2{}_p = 0.04$). Post hoc tests revealed one difference between conspecific and empty area first entry at 140 dph (5.46 versus 13.58 s; $p < 0.0001$) and showed that the time of first entry into the conspecific area tended to decrease at the adult stage (with 16 dph (32.43 s), 60 dph (26.44 s) and 140 dph (5.46 s)) but was not significant. The first entry into the empty area didn't show any significant changes with age (26.23 s at 16 dph, 25.19 s at 60 dph and 13.58 s at 140 dph).

3.2.3. Number of entries into each area

Number of entries into each box (Fig. 23 and Sup Table 1) did not show any differences except those relating to the sex of fish ($F = 9.17$; $p = 0.003$, $n^2{}_p = 0.04$) and in fish ($F = 1.64$, $p = 0.005$, $n^2{}_p = 0.29$). Post hoc test revealed that males were different from females ($p = 0.007$) and from immature fish ($p = 0.026$). Because of this, we analyzed separately the differences at 140 dph between males and females. In this second analysis, GLM showed a sex effect ($F = 11.54$, $P = 0.001$, $n^2{}_p = 0.12$) and an area effect ($F = 10.91$; $p = 0.001$, $n^2{}_p = 0.11$) and a fish effect ($F = 2.72$, $p < 0.0001$, $n^2{}_p = 0.47$). Fig. 23 showed an increase between number of entries into conspecific and empty areas for females versus males (3.13 versus 1.8 entries on average). Females entered into areas significantly more times than males did($p = 0.001$).

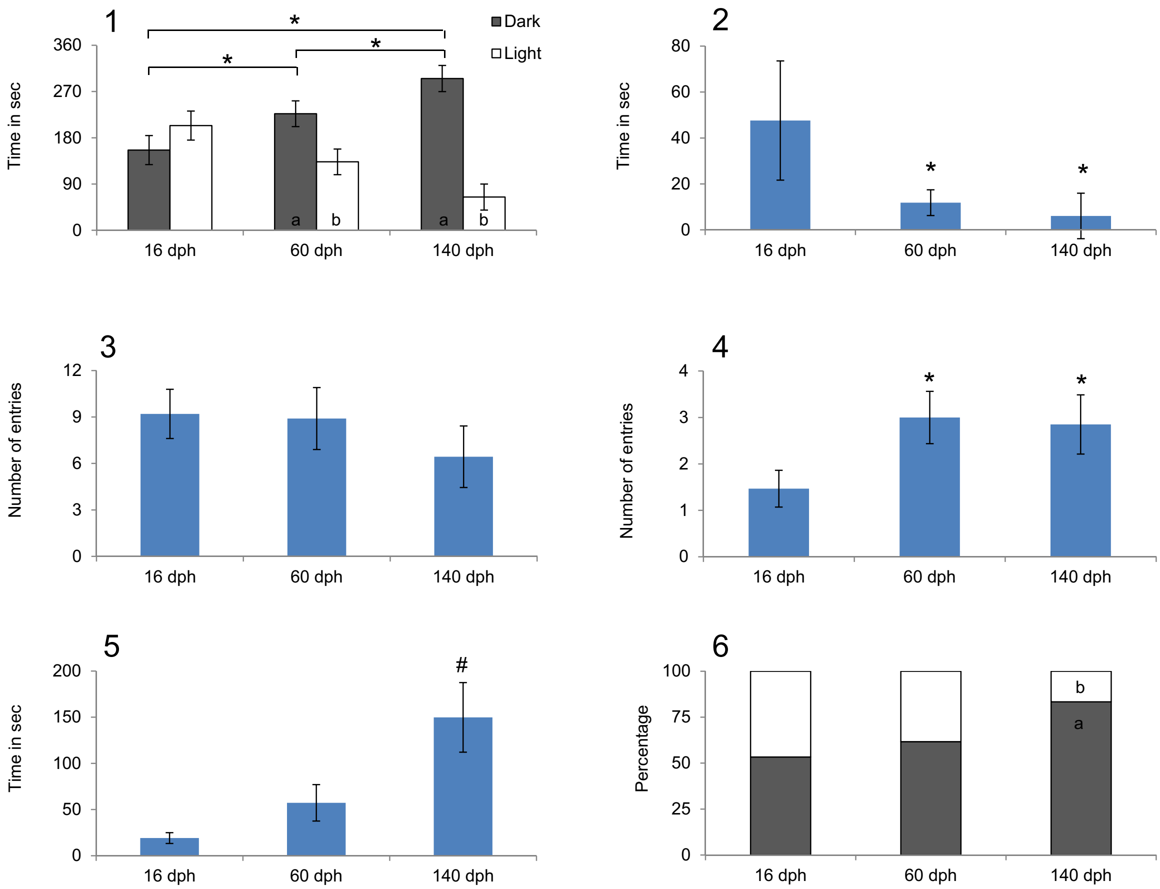

Fig. 1. Results of the light-dark box test with fathead minnows of different ages (16, 60, and 140 dph).
The test was 6 min (360 s) in duration. N = 30 larvae at 16 dph, 60 juveniles at 60 dph, and 60 adults (30 females and 30 males) at 140 dph. (1) Time spent (in sec) in dark and light areas during the test. (2) Time of dark first entry (in sec). (3) Total number of entries into dark area. (4) Number of entries in the first minute in dark. (5) Mean time (in sec) spent in dark area. (6) Place at the end of the test (in percentage). Letters indicate significant differences between time spent in the dark and light areas at each age (larvae, juvenile and adult) with $p < 0.05$. Asterisks indicate significant differences in the time spent in the dark area between larvae, juvenile and adult for $p < 0.05$ and # for $p < 0.1$. Error bars show 95% Confidence Intervals.

3.2.4. Mean time spent in each area

The mean time spent in the conspecific area (Fig. 24 and Sup Table 1) was calculated as the total time in the conspecific area divided by the number of entries into this area. The mean time spent in the empty area was calculated in a similar way. GLM showed an area effect ($F = 139.12$; $p < 0.0001$, $n^2_p = 0.37$) and an interaction between age and area ($F = 6.06$; $p = 0.003$, $n^2_p = 0.05$). The mean time in the conspecific area was significantly greater than the mean time in the empty area in larvae (194 versus 37 s), juveniles (166 versus 52 s), and adults (224 versus 8 s) ($p < 0.0001$ for all three comparisons). The mean time in the conspecific area tended to increase between 60 dph (166 s) and 140 dph (224 s; $p = 0.058$).

3.2.5. Place at the end

There was a significant effect for area at the end of the test ($F = 218.75$; $p < 0.0001$, $n^2_p = 0.53$) and interaction between area and age ($F = 9.70$; $p < 0.0001$, $n^2_p = 0.09$). At each age, fish finished more in the conspecific box ($p < 0.0001$ for all). We observed a slight preference for the conspecific area at the end of the test that increased in adults (Fig. 25 and Sup Table 1). At 16 and 60 dph, 77% and 70% of fish finished their test in the conspecific area, respectively, whereas 97% were with conspecifics at the end of the test 140 dph. Adults finished more in the conspecific box than juveniles ($p = 0.0003$).

4. Discussion

Fewer larvae were investigated in our tests than juveniles and adults, as the available time for assessment of larval-stage was affected by another experiment. With the juveniles and the adults, we performed these test on two or three days (for example at 59, 60 and 61 days post hatch in juveniles) which allowed more time for assessment of the fish at these stages. The lower n for the larval stages is a potential limit of the current study. However, we feel the trends in behavior changes with age of FM are consistent and repeatable enough for us to draw conclusions from our data, regardless of the lower n for the larval stage fish.

4.1. Light-dark box test

Fish showed a preference for the light area at the larval stage and a preference for the dark area starting at 60 dph. After that there was an increase in dark preference with age and a decrease in time spent in the light area in adult fathead minnows. Studies with adult zebrafish found varying results in tests of light-dark preference. Gerlai et al. (2000) showed zebrafish had a clear preference for light whereas Serra et al. (1999) found a preference for dark. A third study investigated these differences (Stephenson et al., 2011) and established that the light-dark side preference is determined by light intensity. In that study, zebrafish preferred the light side when the intensity was under 4.10^{17}/s/m^2 photons (≈200 Lux), and preferred the dark side when the intensity was over 4.10^{17}/s/m^2

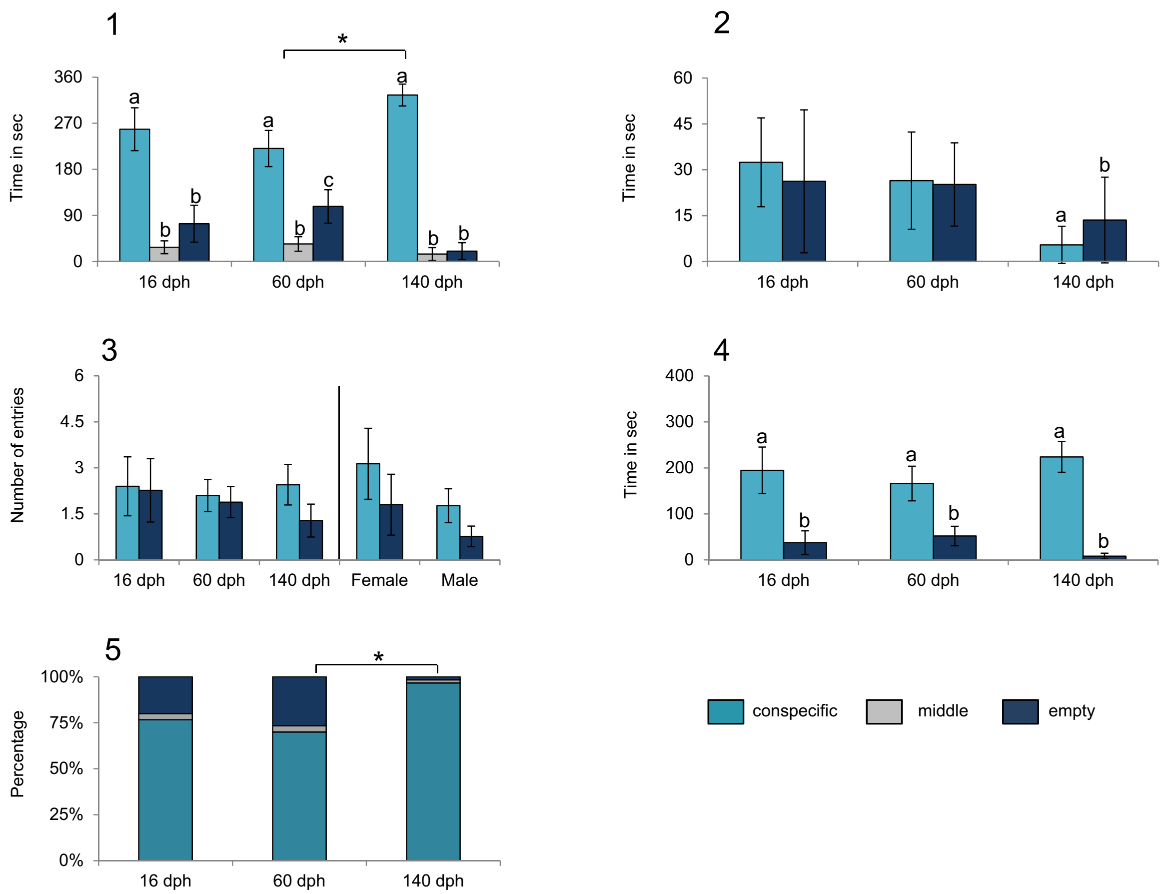

Fig. 2. Results of the social preference test in fathead minnows at 16, 60, and 140 dph.
The test was 6 min (360 s) in duration. N = 30 larvae at 16 dph, 60 juveniles at 60 dph, and 60 adults (30 females and 30 males) at 140 dph. (1) Time spent (in sec) in conspecific, middle and empty areas during the test. (2) Time of first entry into the conspecific and empty area (in sec). (3) Total number of entries into the conspecific and empty areas. (4) Mean time (in sec) spent in conspecific and empty areas. (5) Place at the end of the test (as a percentage). Letters indicate significant differences between time spent in the conspecific, middle and empty areas at each age of fish (larvae, juvenile and adult) with $p < 0.05$. Asterisks indicate significant differences in time spent in the conspecific area comparing the different ages of fish (larvae, juvenile and adult) with $p < 0.05$. Error bars show 95% Confidence Intervals.

photons. In our case, the light intensity in the current fathead minnow study was quite high (450 Lux) and so the place preference for dark seems to agree with the results of Stephenson et al. (2011).

Some other studies used a light-dark box to test zebrafish adults between 150 and 240 dpf. Grossman et al. found an average of 40 s spent in the light area (Grossman et al., 2010) and Cachat et al. found an average of 100 s in the light area (Cachat et al., 2013). These two studies placed the fish in the dark part at the beginning of the test, whereas we placed it in the light. A third study with zebrafish found an average time in the light area that was closer to our results with fathead minnows (around 60 s for them and 64 s for us) but there was no information given on the place of the zebrafish at the beginning of their test (Stewart et al., 2010).

The light-dark preference test was recently validated as an anxiety test using drugs with known effects (like ethanol and fluoxetine, for example) in adult zebrafish (Magno et al., 2015). The results showed differences in the time spent in the dark area after acute immersion in a drug. These behavioural responses to known toxicants would be very interesting to study with fathead minnow as well.

The results obtained in our study, of an increase in dark preference with age of fathead minnow, could be explained by the fish moving to the "safest" area. Fathead minnow are a prey species that live in different places (in clear water or in turbid water). However, they usually live in areas with a lot of hard objects or vegetation that may provide darkness and areas to hide from predators (Holzman, 2014). It's known that vision is very important in prey detection in light water (Abrahams, 2005) and so hiding in the darker areas may protect the minnows from predators.

4.2. Social test

At each age we tested, fathead minnow preferred to be with conspecifics but this preference was stronger at the adult stage than at larval stage. The time of first entry into the conspecific area reduced with age (but first entry into the empty area stayed the same on average). No differences in the number of entries were observed among the three ages, but differences were found between males and females at 140 dph. Females moved more than males, but both entered more frequently into the conspecific area than into the empty area. The time spent in the conspecific area between juveniles and adults increased, and adults stayed more in this area when they entered it. But because we put male conspecifics when females were tested and female conspecifics when males were tested, the change in behavior observed in the test might be due to the change in sexual behavior due to the emergence of sexual traits in fish.

Adult fathead minnows' social cohesion was known previously (Chivers et al., 1995; Yunker et al., 1999). This fish naturally prefers to live in groups. It is known that fathead minnow are able to discriminate one conspecific from another one (Brown and Smith, 1994). In this study, fish from the same cohort, but not from the same aquarium, were chosen as conspecifics.

In our test, adults spent an average of 325 s (of the 360 s assessment time) in the conspecific area. We had five fish in the conspecific area and this probably resulted in a stronger response, like in tests with zebrafish (Gerlai et al., 2000) and guppies (*Poecilia reticulata*) (Bisazza et al., 2010). Gerlai et al. showed a large difference in the time spent near conspecifics depending on the number of conspecific fish. Zebrafish spent on average 54 s near conspecifics when 1–4 fish were in the conspecific area. But when 5 fish were used as conspecific fish, the test zebrafish spent on average 234 s near their conspecifics. Gerlai et al. (2000) used younger fish than us (between 90 and 120 dpf) which could explain some of the differences in time spent near conspecifics between these two studies. Generally it appears that the number of fish in the conspecific area seems to be important in other fish species (zebrafish and guppies) and possibly in fathead minnows, although we did not specifically tests this.

Three other studies analyzing socialization in fish have tested the influence of the number of conspecific fish. One study tested zebrafish between 150 and 240 dpf (older than our fish) but used just one fish as the conspecific. Under these conditions zebrafish spent an average of 200 s near the one conspecific (Pham et al., 2012). In studies of the effects of LSD on zebrafish behaviour, there was an average of 170 s spent near one conspecific for control fish (Grossman et al., 2010). In another study of the effects of ibogaine (a plant-derived psychoactive substance) on zebrafish, the control fish spent on average 120 s in the conspecific area (Cachat et al., 2013). Overall, the reduction of time spent near one conspecific compared to five conspecifics seems to be around 20% of the total time, even if older fish are used. The importance of conspecific number on the results was also shown in other fish species. Angelfish preferred the side with 4 conspecific fish compared to the side with only one (Gómez-Laplaza and Gerlai, 2011).

Few studies have investigated how behavior changes as fish mature and age. In our study of fathead minnow, we showed an increase of conspecific preference with age. Ledesma and McRobert (2008) examined the behaviour of juvenile guppies in 10, 30 and 50 day-old fish. They showed a preference for conspecific at each stage. Their 10 and 30 day-old fish showed "basic shoaling behavior", but 50 day-old fish demonstrated "shoaling discrimination" like in adults. Dreosti et al. (2015) analyzed the development of social behavior in zebrafish from 1 to 3 weeks of age. They showed that there was a maturation of social behaviour with the development of the visual system, and proved that certain drugs could affect social maturation. Buske and Gerlai (2011) investigated the increase of shoaling preference with age in zebrafish, and showed that inter-individual distance between fish strongly decreased with age. A study with guppies investigated maturation of shoaling with age (Miletto Petrazzini et al., 2012) and found similar results to ours study; specifically, an increase in guppies' preference for conspecifics as they age (from the larval stage to the adult stage). Another recent study also demonstrated a decrease in inter-individual distance with age in zebrafish (Mahabir et al., 2013) between 7–87 dpf and showed an alteration of this behavior after embryonic exposure to alcohol. This inter-individual distance test could be used with fathead minnow for toxicological assessments in the future. We have demonstrated that similar social behaviours are observed with fathead minnows as seen with various studies of zebrafish, and this new inter-individual distance test would permit the detailed exploration of shoaling, and would increase the suite of behavioural tests available for fathead minnows.

Shoal cohesion and distance between individuals is very important for survival of some fish species. Chivers et al. (1995) studied shoal cohesion in fathead minnow in the lab, and found that groups of familiar fish survived better than groups of unfamiliar fish against a predator because the cohesion was stronger.

4.3. Conclusion

We clearly demonstrated a maturation of behavior in fathead minnow using a light-dark box test and a social preference test. In both tests, preferences for socializing were already present in larvae but were very much more consistent at the adult stage. Preferences for dark were weak in the larval stage fish, and grew stronger as the fish became juveniles and adults. The fathead minnow is another good model fish (along with zebrafish) to investigate these kinds of behaviors. This study helps define preferences for light-dark and socializing that occur as fathead minnow age, and helps to define the appropriate ages for behavioral studies with fathead minnows.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.beproc.2017.02.004.

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