



Visible Implant Elastomer tagging influences social preferences of zebrafish (*Danio rerio*)

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Abstract

Identification of individuals is a prerequisite in many behavioural studies. Visible Implant Elastomer (VIE) colour tags are a well-established way to mark animals. VIE tagging does not seem to affect individual growth or survival. However, studies verifying their neutrality during social interactions are less common. Here, individual male and female zebrafish *Danio rerio* were simultaneously given the choice between two shoals, each consisting of six fish. Members of one shoal were uniformly marked with one VIE tag of a given colour, whereas the other shoal was sham-tagged. In total, 10 different colours were used. Test fish spent significantly more time near the tagged shoal (56%) than near the sham-tagged shoal (44%). Tag colour did not significantly influence the preferences. The results highlight the importance of confirming the neutrality of colour tags before using them in behavioural studies. Especially, our study advises caution when using marked and unmarked fish simultaneously.

Keywords

shoaling, schooling, artificial marks, social behaviour, VIE tags.

1. Introduction

Identification of single individuals within groups is often required in behavioural, evolutionary and ecological studies (Herbinger et al., 1990; Godin et al., 1996; Croft et al., 2005). This is the case, for example, when single individuals within a group need to be continuously monitored or when individuals originating from different populations or strains need to be kept

together within the same tank. As individuals frequently feature very similar phenotypes, reliable discrimination is often impossible — at least for the human observer.

Tagging of animals is a possibility to solve this problem. Accordingly, there are several methods to mark individuals, ranging from chemical to mechanic or electronic-based approaches (Sire et al., 2000; Leips et al., 2001; Webster & Laland, 2009; Hohn & Petrie-Hanson, 2013). Their applicability depends on several factors. External tags like spine bands, dart tags, painted marks, or spine clips are cheap, but their reliability can be reduced because marks might be lost (e.g., in case of spine bands) (Håstein et al., 2001). Sire et al. (2000) proposed a marking technique based on scale regeneration in zebrafish *Danio rerio* Hamilton (1822). Although this method is cheap as well, a problem is the inability to recognize a regenerated scale from a distance, which makes it unsuitable for behavioural observations. Furthermore, similar scale patterns might occur naturally (e.g., loss of scales due to intra-specific fights or predator attacks). Electronic tags like passive integrated transponders (PIT) are very reliable but more costly (Cousin et al., 2012), and they require a certain minimal body size (Ferrari et al., 2014).

A frequently used way to mark fish is the injection of colourful substances. For example, Alizarin red S, calcein, or Alcian blue were used to tag guppies *Poecilia reticulata* Peters (1859), Atlantic salmon *Salmo salar* L., killifish *Heterandria formosa* Girard (1859) and cichlids (Taborsky, 1984; Leips et al., 2001; Bashey, 2004). However, these colours might fade with time (Bashey, 2004). One of the most efficient methods to mark fish are Visible Implant Elastomers (VIE) (NMT INC Northwest Marine Technology), which consist of a two-part silicone based material. VIE tags are injected as a liquid that cures into an elastic, biocompatible solid. They are available in several fluorescent and non-fluorescent colours. The fluorescent colours are highly visible under ambient light. Thus, they are highly suitable for recognition and monitoring of individuals under natural conditions. Many studies verified that VIE tags have no negative effect on fish survival or well being (e.g., Dewey & Zigler, 1996; Frederick, 1997; Reeves & Buckmeier, 2009). However, although it is generally assumed that VIE tagging does not affect behavioural interactions between individuals, there exist only few studies proving this claim (e.g., Croft et al., 2004). Generally, colour tags might influence behaviour in different ways. For example, as tagged individuals look different they might be avoided by con-specifics due to oddity effects

(Landeau & Terborgh, 1986). Furthermore, many fish parasites are colourful (Wittrock et al., 1991; Noga, 1996). Thus, novel colour patterns might be confused with ectoparasites or diseases and thus VIE-tagged individuals might be avoided (Rosenqvist & Johansson, 1995). In contrast, the addition of artificial colour marks might increase the attractiveness of individuals as mating partners, as has been shown in birds (Witte & Sawka, 2003).

The zebrafish is a common vertebrate model species in fields like developmental biology and genomics (Lieschke & Currie, 2007; Kettleborough et al., 2013). It possesses a tetrachromatic colour vision, with ultraviolet, blue, green and red peak absorbances (see, for example, Fleisch & Neuhaus, 2006; Spence & Smith, 2008; and citations therein). In recent years, zebrafish have garnered increasing interest also from behavioural and evolutionary science (Spence et al., 2008; Blaser & Vira, 2014). Here, many studies focus on their shoaling (Gerlach & Lysiak, 2006; Pagnussat et al., 2013; Qin et al., 2014) and reproductive behaviour (Spence & Smith, 2005; Skinner & Watt, 2007; Hutter et al., 2010, 2011). As zebrafish appear rather uniform for the human observer, it would be useful to mark them individually. The goal of our study was to elucidate whether VIE tags affect the shoaling behaviour of zebrafish. To answer this question male and female zebrafish were given the dichotomous choice between a shoal consisting of colour tagged individuals and a shoal consisting of sham-tagged individuals. As different colours might elicit different responses 10 different coloured VIEs were used in total.

2. Material and methods

2.1. Experimental subjects

Zebrafish used in this study were the third and fourth generation offspring of wild-caught fish obtained from a branch of River Brahmaputra in the Assam region of India (latitude ca. 26°N and longitude ca. 90°E). Fish were kept in tanks containing between 50 and 400 l of tap water (temperature $28 \pm 1^\circ\text{C}$, light regime of 12L:12D), which was cleaned and aerated through internal filters. Water was partly exchanged weekly. Tanks were separated from each other by green opaque partitions. Thus, fish in each tank were familiar to each other but had no opportunity to get in contact with other groups. The fish were fed 3 times a day in excess on a mix of living *Artemia* sp. nauplii, *Chironomus* sp. larvae and flake food. Only adult fish, aged between 1 and 2 years, were used in the experiments.

2.2. Experimental design

Fish were tested between March and September 2009. Zebrafish's main spawning time is in early morning within the first two hours of day (Hutter et al., 2010). To minimize the influence of sexual behaviour, experiments started at least 3 h after lights were turned on. At least 24 h before the start of the respective trials, approx. 40 fish for each colour were caught from their holding tanks and haphazardly divided into two groups. Fish of one group were tagged using VIE tags while the other half was sham-tagged. To tag and sham-tag the fish we anesthetised them using MS222 (Tricaine). Then, the VIE tag was injected under the skin dorsally close to the head using a syringe needle (Figure 1). In the sham-tagged group the syringe needle was inserted like in the tagged group, but no colour was injected. Consequently, groups differed only in the absence or presence of a VIE tag. Both, tagging and sham-tagging was done by the same two persons (SH and CAS) to ensure that potential results were not influenced by individual tagging techniques. Ten different colours (black, blue, brown, green, orange, pink, purple, red, white and yellow) were used. Brown, black, white and purple VIE tags do not fluorescent, while the other colours do. Responses to the non-fluorescing colours did not differ significantly from response to the fluorescing tag colours (GLM, $N_{\text{non-fluorescing}} = 50$, $N_{\text{fluorescing}} = 70$, $F = 0.299$,

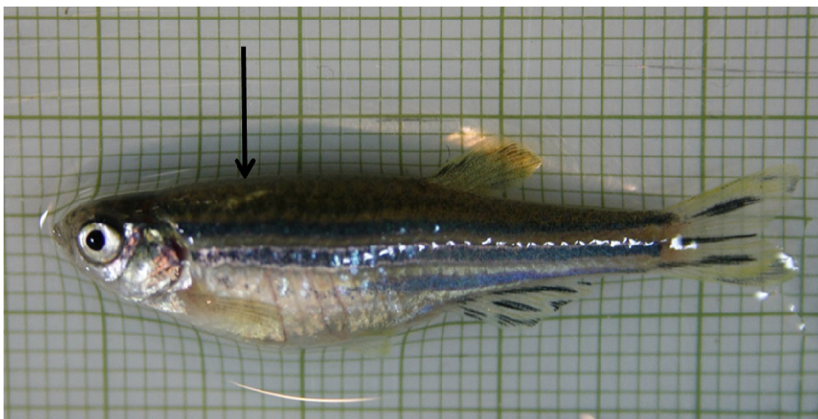


Figure 1. Stimulus fish marked with a white VIE tag (below arrow). VIE tags were injected under the skin dorsally close to the head, where they were not overly conspicuous. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/1568539x>.

$p = 0.567$). To differentiate between potential effects of the different colours each shoal was uniformly colour-marked. After the fish were treated they were kept in treatment-sorted groups of about 20 fish in 45 l tanks under the same conditions as described above.

Shoaling behaviour was tested in a classical binary choice paradigm (e.g., Engeszer et al., 2004; Wright & Krause, 2006; Frommen et al., 2007), which consisted of a tank measuring $80 \times 35 \times 40$ cm. The tank was divided into three compartments: two stimulus compartments on the right and left side (measuring 20 cm each), and a test fish compartment (measuring 40 cm) in the middle. Compartments were separated by perforated, clear Plexiglas. This enabled the test fish to have visual and olfactory contact to each of the two stimulus groups. An association zone was marked 10 cm in front of each stimulus compartment on the front and back wall using a black permanent marker, leaving a neutral zone of 20 cm in the middle. Before each trial the tank was filled with aged tap water ($26 \pm 2^\circ\text{C}$). A 30-W fluorescent tube placed 56 cm above the tank illuminated the set-up. Interactions of the fish with the environment outside the tank were prevented by tightening a black curtain around the test tank.

At least 24 h after tagging and sham-tagging took place, all fish were checked for abnormal behaviours. All fish showed normal swimming behaviour and no signs of stress caused by the tagging procedure. Then, six haphazardly chosen tagged fish were placed in one outer compartment and six sham-tagged in the other. As sex has been shown to influence shoaling preferences of zebrafish (Gerlach & Lysiak, 2006) each group was composed of 3 males and 3 females. The position of the tagged shoal alternated between tests to control for potential side effects. The test fish was caught haphazardly from one of the holding tanks. Test fish and stimulus fish always came from different tanks, as familiarity has an impact on shoaling decisions in zebrafish (Gerlach & Lysiak, 2006). The test fish was put in a transparent plastic cylinder (diameter 20 cm), which was placed in the middle of the tank. After acclimatisation for two minutes the cylinder was lifted from behind the curtain by a string and the test fish was allowed to sample both shoals for 15 min. Thereafter, the test fish's behaviour was recorded for 15 min using a webcam, placed in front of the tank and connected to a computer behind the curtain. At the end of a trial the standard length (SL) and body mass of all fish were measured. From these values the body condition factor (BC) was calculated following Bolger & Connolly (1989). Tagged and

sham-tagged shoals did not differ significantly in mean SL \pm SD (tagged: 2.56 ± 0.29 cm; sham-tagged: 2.59 ± 0.28 cm; Wilcoxon matched pairs signed ranks test, $N = 120$, $V = 2056.5$, $p = 0.616$), BC (tagged: 1.97 ± 0.22 ; sham-tagged: 1.95 ± 0.21 , Wilcoxon matched pairs signed ranks test, $N = 120$, $V = 2527$, $p = 0.186$) and mean absolute size difference to the test fish (tagged: 0.2 ± 0.21 cm; sham-tagged: 0.2 ± 0.20 cm, Wilcoxon matched pairs signed rank test, $N = 120$, $V = 2231.5$, $p = 0.455$). Afterwards, shoal fish were placed in different tanks where they remained until they were used in further trials. Focal fish (58 males, 62 females) were used only once and did not serve as shoal fish. Approximately 400 fish (200 tagged, i.e., 20 fish per colour and 200 non-tagged) were used to create 240 unique shoals (120 tagged and 120 non-tagged shoals), i.e., shoal fish were repeatedly used but never on the same day or in the same combination. This was done in order to reduce the number of experimental animals, following animal welfare guidelines and the framework of the '3Rs' (Replacement, Reduction, Refinement) (Russel & Burch, 1959). In total, 120 trials (black tags $N = 11$, blue tags $N = 11$, red tags $N = 14$, brown tags $N = 10$, green tags, $N = 10$, orange tags $N = 13$, pink tags $N = 10$, purple tags $N = 19$, white tags $N = 10$ and yellow tags $N = 12$) were conducted.

2.3. Data analysis

Videos were analysed by one person (CAS) blindly with respect to tagging status of the stimulus shoals using Observer XT (Version 7.0). The time the test fish spent in the association zones near the stimulus shoals was measured. We calculated the proportion (in percent) of time the test fish spent in the association zones near the colour-tagged group and the sham-tagged group (i.e., $\text{proportion}_{\text{tagged}} = \text{time}_{\text{tagged}} / (\text{time}_{\text{tagged}} + \text{time}_{\text{sham-tagged}})$, and vice versa). Data were analysed using R 2.9.1 (R Development Core Team, 2009). Proportional data were analysed using a generalized linear model (glm) with quasibinomial error distribution (as the data showed overdispersion) using the `cbind` option. This feature allows combining two variables, i.e., proportion of time spent with the tagged shoal and the sham-tagged shoal, into one variable, which was the dependent variable. Tag colour and sex of the test fish were added as fixed factors and SL and BC of test fish as well as the mean difference in SL and BC between the two stimulus shoals (averaged value of the tagged shoal minus averaged value of the sham-tagged shoal) were included as covariates. To examine size-assortative preferences,

we calculated the relative absolute size difference between test and shoal fish as follows: $|\text{SL of test fish} - \text{average SL of tagged shoal}| - |\text{SL of test fish} - \text{average SL of sham-tagged shoal}|$. This index was included as covariate as well. Tests of statistical significance were based on likelihood ratio tests (LRT) which follow an F -distribution. Non-significant factors were removed from the models in order of statistical relevance. Thus, reported p -values of models refer to the increase in deviance when the respective variable was removed. The intercept model indicates whether preferences deviate from random expectation, i.e., 50%. Probabilities are two-tailed throughout.

3. Results

Test fish spent on average 88.58% (average \pm SE 797 ± 10.69 s) of the experimental time in the choice zones near the shoals. They spent on average 55.92% ($\pm 2.95\%$ SE) of this time near the shoals composed of colour tagged fish (glm, intercept, $t_{119} = 2.431$, $p < 0.017$, Figure 2). The respective tag colour did not significantly affect shoal choice ($p = 0.48$, Table 1; average

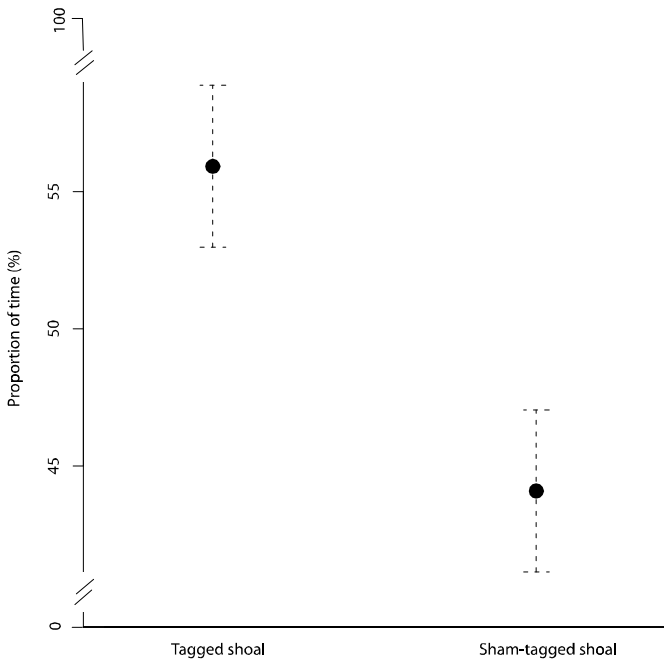


Figure 2. Average percentage of time \pm SE test fish spent with the tagged and sham-tagged shoal.

Table 1.

Results of the glm analysing the effects of tag colour, sex, standard length (SL) and body condition (BC) of the test fish (TF).

Explanatory variable	<i>F</i>	<i>p</i>	Estimate	SE
Sex	0.419	0.519	0.107	-0.297
Tag colour	0.956	0.480	-	-
SL test fish	0.350	0.556	-0.181	0.489
BC test fish	<0.001	0.985	0.153	0.456
Diff. BC shoals	1.619	0.206	0.888	0.752
Diff. SL shoals	7.407	0.008	1.161	0.720
Diff. SL TF to shoals	<0.001	0.989	-0.011	0.822

Relative absolute size differences between TF and shoal fish (see Methods for definition), as well as the effects of differences in SL and BC between the shoals on preference for the shoal consisting of tagged fish.

time near the tagged shoal in percent \pm SE for each colour: blue, $52.30 \pm 11.60\%$; brown, $55.53 \pm 10.98\%$; yellow, $65.87 \pm 7.97\%$; green, $62.24 \pm 9.19\%$; purple, $28.37 \pm 8.39\%$; orange, $57.59 \pm 10.05\%$; pink, $63.01 \pm 9.80\%$; red, $63.57 \pm 8.98\%$; black, $51.43 \pm 9.46\%$; white, $73.96 \pm 9.82\%$). Neither sex, SL or BC of the test fish nor BC differences between shoals or relative absolute size difference between test and shoal fish did significantly affect shoal choice (all $p > 0.2$, see Table 1 for details). However, random SL differences between the two stimulus shoals influenced the preferences ($p = 0.008$, Table 1), indicating that the shoal comprising bigger individuals received greater attention.

4. Discussion

VIE tagging is a cheap and easy method to individually mark fish (Hohn & Petrie-Hanson, 2013) and other animals (Sapsford et al., 2014). However, changes in behavioural responses towards tagged individuals might pose a problem, but did not receive much attention thus far. The results of the current study show that zebrafish discriminated between a colour-tagged shoal and a sham-tagged shoal. This result is in contrast to a study on guppies (Croft et al., 2004), that did not find a significant effect of VIE marking on shoaling preferences, underlining the necessity to validate the use of colourful VIE tags in each species that they are used. The results are in accordance with studies showing that differences in natural colour characteristics between shoals influence individual's shoal choice (e.g., Modarressie et al.,

2006; Kelley et al., 2012). Using pigment mutants Engeszer et al. (2004) showed that zebrafish imprint on the pigmentation patterns of their rearing mates resulting in assortative shoaling preferences for individuals with similar pigmentation. A similar result was found in mollies *Poecilia latipinna* Lesueur (1821), which preferred shoals consisting of individuals featuring similar colouration to their own or to that of their rearing partners (Ledesma & McRobert, 2008). Interestingly, test fish of the present study, that were not tagged themselves and that grew up with non-tagged individuals, preferred the tagged phenotypes.

One difference between our and the aforementioned studies is that we created artificial phenotypic colour patterns which possibly piques fish's curiosity. A potential reason for this might be that novel colour patterns might increase the attractiveness of the tagged fish as mating partners (Farr, 1977; Hughes et al., 2014). However, the trials were conducted outside the usual courtship times of zebrafish. Further, the results revealed no significant influence of the test fish's sex on shoaling preferences, making sexual attraction as explanation rather unlikely. A further explanation for the preference for the tagged shoal might be an increase in aggression towards the tagged fish. Indeed, adding artificial colouration led to a change in aggressive interactions, for example, in vervet monkeys *Chlorocebus pygerythrus* Cuvier (1821) (Gerald, 2001). In studies on the impact of colouration on aggressive interactions of fish, usually the natural aggressive colouration of the respective species is manipulated (Rowland, 1999; Rick & Bakker, 2008). There, an increase of the natural colouration often led to an increase in aggressive behaviour towards the manipulated fish (Rowland, 1999). This increase is usually represented by an increased amount of time spent close to the respective stimulus (Rick & Bakker, 2008; Balzarini et al., 2014). However, if a comparable effect would also account for our findings one would expect stronger preferences for colours which naturally occur in zebrafish. Thus, one might expect a stronger response towards fish that bore yellow, blue or whitish tags. However, the differences between the colours — including the differences between fluorescing and non-fluorescing tag colours — were statistically not significant. Thus, the question of whether some VIE tag colours are more suitable than others when marking zebrafish cannot be definitely clarified with our study and should be elucidated in more detail in the future.

Additionally, the shoal choice of the test fish was influenced by subtle, random mean SL differences between the two stimulus shoals. Larger shoals

appeared to be more attractive than smaller ones. Interestingly, this preference was not influenced by the SL of the test fish itself, indicating that test fish did not shoal size-assortative. However, tagged and sham-tagged stimulus shoals did not systematically differ in mean SL. Thus, preferences for larger shoals cannot explain our finding, that test fish preferred tagged shoals over non-tagged ones. Still, it underlines the fact, that social decisions in general are based on a range of different factors, which might interact with each other (see, for example, Croft et al., 2005; Frommen et al., 2009; Miller et al., 2013).

In summary, colour tagged shoals appeared to be more attractive than non-tagged ones. Thus, using both tagged and non-tagged individuals in a study might systematically confound results, because interactions between fish are not independent from marking. Especially, examination of zebrafish social behaviours could be impaired by this effect. A solution to this problem could be marking all fish in a group, so that behavioural changes towards tagged sub-groups cannot be established. The results highlight the importance of confirming the neutrality of colour tags before using them in behavioural studies.

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References

- Balzarini, V., Taborsky, M., Wanner, S., Koch, F. & Frommen, J.G. (2014). Mirror, mirror on the wall: the predictive value of mirror tests for measuring aggression in fish. — *Behav. Ecol. Sociobiol.* 68: 871-878.

- Bashey, F. (2004). A comparison of the suitability of alizarin red S and calcein for inducing a nonlethally detectable mark in juvenile guppies. — *Trans. Am. Fish. Soc.* 133: 1516-1523.
- Blaser, R.E. & Vira, D.G. (2014). Experiments on learning in zebrafish (*Danio rerio*): a promising model of neurocognitive function. — *Neurosci. Biobehav. Rev.* 42: 224-231.
- Bolger, T. & Connolly, P.L. (1989). The selection of suitable indices for the measurement and analysis of fish condition. — *J. Fish Biol.* 34: 171-182.
- Cousin, X., Daouk, T., Péan, S., Lyphout, L., Schwartz, M.-E. & Bégout, M.-L. (2012). Electronic individual identification of zebrafish using radio frequency identification (RFID) microtags. — *J. Exp. Biol.* 215: 2729-2734.
- Croft, D.P., James, R., Ward, A.J.W., Botham, M.S., Mawdsley, D. & Krause, J. (2005). Assortative interactions and social networks in fish. — *Oecologia* 143: 211-219.
- Croft, D.P., Krause, J. & James, R. (2004). Social networks in the guppy (*Poecilia reticulata*). — *Proc. Roy. Soc. Lond. B: Biol. Sci.* 271: S516-S519.
- Dewey, M.R. & Zigler, S.J. (1996). An evaluation of fluorescent elastomer for marking bluegills in experimental studies. — *Prog. Fish-Cult.* 58: 219-220.
- Engeszer, R.E., Ryan, M.J. & Parichy, D.M. (2004). Learned social preference in zebrafish. — *Curr. Biol.* 14: 881-884.
- Farr, J.A. (1977). Male rarity or novelty, female choice behaviour, and sexual selection in the guppy, *Poecilia reticulata* Peters (Pisces: Poeciliidae). — *Evolution* 31: 162-168.
- Ferrari, S., Chatain, B., Cousin, X., Leguay, D., Vergnet, A., Vidal, M.-O., Vandeputte, M. & Bégout, M.-L. (2014). Early individual electronic identification of sea bass using RFID microtags: a first example of early phenotyping of sex-related growth. — *Aquaculture* 426: 165-171.
- Fleisch, V.C. & Neuhauss, S.C.F. (2006). Visual behavior in zebrafish. — *Zebrafish* 2: 191-220.
- Frederick, J.L. (1997). Evaluation of fluorescent elastomer injection as a method for marking small fish. — *Bull. Mar. Sci.* 61: 399-408.
- Frommen, J.G., Mehli, M., Brendler, C. & Bakker, T.C.M. (2007). Shoaling decisions in three-spined sticklebacks (*Gasterosteus aculeatus* L.) — familiarity, kinship and inbreeding. — *Behav. Ecol. Sociobiol.* 61: 533-553.
- Frommen, J.G., Hiermes, M. & Bakker, T.C.M. (2009). Disentangling the effects of group size and density on shoaling decisions of three-spined sticklebacks (*Gasterosteus aculeatus*). — *Behav. Ecol. Sociobiol.* 63: 1141-1148.
- Gerald, M.S. (2001). Primate colour predicts social status and aggressive outcome. — *Anim. Behav.* 61: 559-566.
- Gerlach, G. & Lysiak, N. (2006). Kin recognition and inbreeding avoidance in zebrafish, *Danio rerio*, is based on phenotype matching. — *Anim. Behav.* 71: 1371-1377.
- Godin, D.M., Carr, W.H., Hagino, G., Segura, F., Sweeney, J.N. & Blankenship, L. (1996). Evaluation of a fluorescent elastomer internal tag in juvenile and adult shrimp *Penaeus vannamei*. — *Aquaculture* 139: 243-248.
- Håstein, T., Hill, B.J., Berthe, F. & Lightner, D.V. (2001). Traceability of aquatic animals. — *Rev. Sci. Tech. OIE* 20: 564-583.

- Herbinger, C.M., Newkirk, G.F. & Lanes, S.T. (1990). Individual marking of Atlantic salmon — evaluation of cold branding and jet injection of alcian blue in several fin locations. — *J. Fish Biol.* 36: 99-101.
- Hohn, C. & Petrie-Hanson, L. (2013). Evaluation of visible implant elastomer tags in zebrafish (*Danio rerio*). — *Biol. Open* 2: 1397-1401.
- Hughes, K.A., Houde, A.E., Price, A.C. & Rodd, F.H. (2014). Mating advantage for rare males in wild guppy populations. — *Nature* 503: 108-110.
- Hutter, S., Penn, D.J., Magee, S. & Zala, S.M. (2010). Reproductive behaviour of wild zebrafish (*Danio rerio*) in large tanks. — *Behaviour* 147: 641-660.
- Hutter, S., Zala, S.M. & Penn, D.J. (2011). Sex recognition in zebrafish (*Danio rerio*). — *J. Ethol.* 29: 55-61.
- Kelley, J.L., Phillips, B., Cummins, G.H. & Shand, J. (2012). Changes in the visual environment affect colour signal brightness and shoaling behaviour in a freshwater fish. — *Anim. Behav.* 83: 783-791.
- Kettleborough, R.N.W., Busch-Nentwich, E.M., Harvey, S.A., Dooley, C.M., de Bruijn, E., van Eeden, F., Sealy, I., White, R.J., Herd, C., Nijman, I.J., Fenyes, F., Mehroke, S., Sc-ahill, C., Gibbons, R., Wali, N., Carruthers, S., Hall, A., Yen, J., Cuppen, E. & Stemple, D.L. (2013). A systematic genome-wide analysis of zebrafish protein-coding gene function. — *Nature* 496: 494-497.
- Landeau, L. & Terborgh, J. (1986). Oddity and the 'confusion effect' in predation. — *Anim. Behav.* 34: 1372-1380.
- Ledesma, J.M. & McRobert, S.P. (2008). Innate and learned shoaling preferences based on body coloration in juvenile mollies, *Poecilia latipinna*. — *Ethology* 114: 1044-1048.
- Leips, J., Baril, C.T., Rodd, F.H., Reznick, D.N., Bashey, F., Visser, G.J. & Travis, J. (2001). The suitability of calcein to mark poeciliid fish and a new method of detection. — *Trans. Am. Fish. Soc.* 130: 501-507.
- Lieschke, G.J. & Currie, P.D. (2007). Animal models of human disease: zebrafish swim into view. — *Nature Rev. Genet.* 8: 353-367.
- Miller, N., Garnier, S., Hartnett, A.T. & Couzin, I.D. (2013). Both information and social cohesion determine collective decisions in animal groups. — *Proc. Natl. Acad. Sci. USA* 110: 5263-5268.
- Modarressie, R., Rick, I.P. & Bakker, T.C.M. (2006). UV matters in shoaling decisions. — *Proc. Roy. Soc. Lond. B: Biol. Sci.* 273: 849-854.
- Noga, E.J. (1996). Fish disease: diagnosis and treatment. — Iowa State University Press, Ames, IA.
- Pagnussat, N., Piato, A.L., Schaefer, I.C., Blank, M., Tamborski, A.R., Guerim, L.D., Bonan, C.D., Vianna, M.R.M. & Lara, D.R. (2013). One for all and all for one: the importance of shoaling on behavioral and stress responses in zebrafish. — *Zebrafish* 10: 338-342.
- Qin, M., Wong, A., Seguin, D. & Gerlai, R. (2014). Induction of social behavior in zebrafish: live versus computer animated fish as stimuli. — *Zebrafish* 11: 185-197.
- R Development Core Team (2009). R: a language and environment for statistical computing. — R Foundation for Statistical Computing, Vienna.

- Reeves, K.S. & Buckmeier, D.L. (2009). Mortality, predation, and tag visibility of fish marked with Visible Implant Elastomer tags. — North Am. J. Fish. Manage. 29: 323-329.
- Rick, I.P. & Bakker, T.C.M. (2008). Males do not see only red: UV wavelengths and male territorial aggression in the three-spined stickleback (*Gasterosteus aculeatus*). — Naturwissenschaften 95: 631-638.
- Rosenqvist, G. & Johansson, K. (1995). Male avoidance of parasitized females explained by direct benefits in a pipefish. — Anim. Behav. 49: 1039-1045.
- Rowland, W.J. (1999). Studying visual cues in fish behavior: a review of ethological techniques. — Environ. Biol. Fish. 56: 285-305.
- Russel, W.M.S. & Burch, R.L. (1959). The principles of humane experimental technique. — Methuen, London.
- Sapsford, S.J., Roznik, E.A., Alford, R.A. & Schwarzkopf, L. (2014). Visible implant elastomere marking does not affect short-term movements or survival rates of the treefrog *Litoria rheocola*. — Herpetologica 70: 23-33.
- Sire, J.Y., Girondot, M. & Babiari, O. (2000). Marking zebrafish, *Danio rerio* (Cyprinidae), using scale regeneration. — J. Exp. Zool. 286: 297-304.
- Skinner, A.M.J. & Watt, P.J. (2007). Strategic egg allocation in the zebra fish, *Danio rerio*. — Behav. Ecol. 18: 905-909.
- Spence, R. & Smith, C. (2005). Male territoriality mediates density and sex ratio effects on oviposition in the zebrafish, *Danio rerio*. — Anim. Behav. 69: 1317-1323.
- Spence, R. & Smith, C. (2008). Innate and learned colour preference in the zebrafish, *Danio rerio*. — Ethology 114: 582-588.
- Spence, R., Gerlach, G., Lawrence, C. & Smith, C. (2008). The behaviour and ecology of the zebrafish, *Danio rerio*. — Biol. Rev. 83: 13-34.
- Taborsky, M. (1984). Broodcare helpers in the cichlid fish *Lamprologus brichardi* — their costs and benefits. — Anim. Behav. 32: 1236-1252.
- Webster, M.M. & Laland, K.N. (2009). Evaluation of a non-invasive tagging system for laboratory studies using three-spined sticklebacks. — J. Fish Biol. 75: 1868-1873.
- Witte, K. & Sawka, N. (2003). Sexual imprinting on a novel trait in the dimorphic zebra finch: sexes differ. — Anim. Behav. 65: 195-203.
- Wittrock, D.D., Bruce, C.S. & Johnson, A.D. (1991). Histochemistry and ultrastructure of the metacercarial cysts of blackspot trematodes *Uvulifer ambloplitis* and *Neascus pyriformis*. — J. Parasitol. 77: 454-460.
- Wright, D. & Krause, J. (2006). Repeated measures of shoaling tendency in zebrafish (*Danio rerio*) and other small teleost fishes. — Nature Protocols 1: 1828-1831.