

# Habitat-dependent olfactory discrimination in three-spined sticklebacks (*Gasterosteus aculeatus*)

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**Abstract** The ability to recognize conspecifics is indispensable for differential treatment of particular individuals in social contexts like grouping behavior. The advantages of grouping are multifarious, and there exist numerous additional benefits of joining aggregations of conspecifics. Recognition is based on different signals and transmitted via multiple channels, among others the olfactory channel. The sensory system or the combination of sensory modalities used in recognition processes is highly dependent on the availability and effectiveness of modalities, which are a function of the environmental conditions. Using F1-generations of six three-spined stickleback (*Gasterosteus aculeatus*) populations from two habitat types (tea-stained and clear-water lakes) from the Outer Hebrides, Scotland, we investigated whether individuals are able to recognize members of their own population solely based on olfactory cues and whether the habitat type an individual originated from had an influence on its recognition abilities. When given the choice (own vs. foreign population) sticklebacks from tea-stained lakes significantly preferred the odor of their own population, whereas fish from clear-water habitats did not show any preference. Moreover, fish from the two habitat types differed significantly in their recognition abilities, indicating that olfactory communication is better developed when visual signaling is disturbed. Thus, the observed odor preferences appear to be the consequence of different selective constraints and adaptations as a result of the differences in environmental conditions that have acted on the parental generations. These adaptations are likely genetically based as the differences are present in the

F1-generation that had been reared under identical laboratory conditions.

**Keywords** Olfactory signals · Lighting environment · Turbidity · Population recognition · Social groups · Fish

## Introduction

Throughout the animal kingdom, the recognition of conspecifics versus heterospecifics, population members versus non-members, familiars versus non-familiars, kin versus non-kin and even the recognition of single individuals has been shown to be important and beneficial, especially in group-living animals (e.g., Krause and Ruxton 2002). In general, the recognition of conspecifics is a process in which animals become familiar with particular individuals, remember them and are able to treat them accordingly (Mateo 2004). This is important in contexts related to social interactions like grouping behavior (e.g., Krause and Ruxton 2002) but also in contexts related to mate-choice decisions and inbreeding avoidance (e.g., Pusey and Wolf 1996). The former has received much attention as many animal species live permanently or temporarily in social aggregations composed of conspecifics (Wright and Krause 2006). In fish, for example, the formation of shoals is very common (Krause and Ruxton 2002), which can be explained by advantages of group members over non-group members (Pitcher and Parrish 1993).

The decision process whether to join a group or to remain solitary is very complex and a multiplicity of factors have to be taken into account (see Pitcher and Parrish 1993; Krause and Ruxton 2002). The influence of familiarity on group-joining decisions has recently received increasing

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interest as it has been shown to enhance the benefits of grouping: advanced foraging efficiency (Griffiths et al. 2004; Ward and Hart 2005; Strodl and Schausberger 2012a), enhanced anti-predator behavior (Chivers et al. 1995; Griffiths et al. 2004; Strodl and Schausberger 2012b), stable dominance ranks within the group (Höjesjö et al. 1998), reduced food competition (Utne-Palm and Hart 2000), better growth (Gerlach et al. 2007), improved information transfer (Swaney et al. 2001) and raised reproductive success (Strodl and Schausberger 2013).

The recognition of conspecifics is based on a wide variety of different signals transmitted via multiple channels and can be acoustic, morphological, tactile, behavioral, visual or olfactory (e.g., Rosenthal and Lobel 2005). The sensory modality or the combination of modalities used is highly dependent on the species, the repertoire of modalities available and the effectiveness of modalities as a function of the environmental conditions (e.g., Candolin 2003). Olfactory cues have been shown to be important in the recognition of conspecifics all over the animal kingdom (reptiles: Dawley 1984; fish: McLennan and Ryan 1997; insects: Singer 1998; mammals: Heth et al. 2003). In aquatic systems, chemical cues are very important due to the properties of water as a solvent and its ability to disperse these cues, while other cues (e.g., visual cues) are used in short-range communication, as vision is strongly influenced by depth, complexity and turbidity of the aquatic habitat (Douglas and Hawryshyn 1990; Ward et al. 2007). Thus, in fish, communication via olfactory cues has been demonstrated to play a crucial role with respect to homing, schooling and shoaling, sibling recognition and the recognition of conspecifics and population members (Olsén 1986; Courtenay et al. 1997; McLennan and Ryan 1997; Behrmann-Godel et al. 2006). The ability to compensate for a restricted use of one sense, for example triggered by a limited visual environment, by the enhanced acuity in another sense (“compensatory plasticity hypothesis”: Rauschecker and Kniepert 1994), is known as “sensory plasticity”. A study by Chapman et al. (2010) on guppies, for example, showed a sensory switch from vision to chemoreception (smell/taste) as response to limited availability of light during rearing.

To sum up, the general advantages of grouping are well-studied (Pitcher and Parrish 1993; Krause and Ruxton 2002) and the decision whether to join a group or not is supposed to be a function of the environmental conditions and/or the efficiency of sensory systems under these conditions; the latter is a research field that is less well explored. Thus, this study aimed to investigate the influence of differential lighting conditions on the ability to recognize population members based on olfactory cues alone in a shoal-choice context. The three-spined stickleback (*Gasterosteus aculeatus*) is a small coldwater fish distributed throughout

various freshwater and marine habitats all over the Northern Hemisphere, which forms large shoals in winter (Bell and Foster 1994). The parental generation used in this study originated from two habitat types, clear-water and tea-stained, i.e., dystrophic lakes, on the island of North Uist, Scotland, which differ strongly in their natural lighting conditions and have evolved independently for about 10,000 generations (Giles 1981). We hypothesized that in a habitat in which the use of visual cues is constrained due to increased turbidity, the use of the olfactory system should become more pronounced and thus olfactory communication might be better developed (see Rauschecker and Kniepert 1994). A study by Ward et al. (2009) confirmed our assumption as wild-caught sticklebacks from two ecologically distinct populations (high water flow/high visibility vs. low water flow/low visibility) also differed in their response to conspecifics based on olfactory cues, however, which olfactory cues were used for recognition remained unknown in this study. To control for confounding environmental effects, we raised and reared the F1-generation of six stickleback populations, three from each habitat type, under standardized laboratory conditions and, at adult (non-reproductive) stage, tested their ability to recognize members of their own populations based on olfactory cues alone.

## Materials and methods

### Experimental subjects

The three-spined sticklebacks originated from six lakes of two habitat types, differing in water turbidity, on the island of North Uist, Scotland: tea-stained lakes (Loch a' Bharpa: 57°34'31,69"N, 7°17'31,74"W; Loch Scadavay: 57°35'01,37"N, 7°13'56,05"W; Loch Tormasad: 57°33'44,10"N, 7°19'12,93"W) and clear-water lakes (Loch Scarie: 57°36'19,63"N, 7°29'49,27"W; Loch Hosta: 57°37'29,57"N, 7°27'59,84"W; Loch Grogary: 57°36'34,85"N, 7°30'01,31"W).

Test fish used in this study were F1-generation offspring (hatching date: June–August 2007) of a parental generation caught in April 2007. In detail, the F1-generation resulted from random within-population crosses of wild-caught fish conducted in glass tanks measuring 50 cm × 30 cm × 30 cm (L × W × H) under standardized laboratory conditions. Breeding and rearing conditions were identical for tea-stained and clear-water fish. In addition, all parental individuals were only used once to avoid pseudoreplication. Tanks of parental individuals were illuminated by fluorescent tubes (TrueLight, Natural Daylight) mimicking natural daylight and were located in an air-conditioned room under standardized summer light regime (16 h light/8 h dark; temperature 17 ± 1 °C).

After a successful spawning event, eggs were left in males' nests (artificial nesting material: 2 g of green threads) during development. As soon as the fry had hatched males were removed from the tanks and the fry were fed daily with *Artemia* nauplii for 10 weeks to excess. The fry were kept in the tanks for 6 weeks and were then moved to plastic tanks (40 cm × 20 cm × 25 cm). At the same time, the maximum capacity of fish per tank was reduced to 20 individuals. The light regime was changed to winter conditions (10 h light/14 h dark) until March 2008, set back to summer conditions again (16 h light/8 h dark) and was finally changed to standard winter conditions (8 h light/16 h dark) in October 2008. The latter represent the light conditions during experiments (see below), which were conducted in December 2008. In total, 26 families were available; tea-stained populations were represented by 11 families (a'Barpa:  $N = 3$ ; Scadavay:  $N = 6$ ; Tomasad:  $N = 2$ ), whereas clear-water populations were represented by 15 families (Scaric:  $N = 6$ , Hosta:  $N = 3$ ; Grogary:  $N = 6$ ). Due to the winter conditions, fish showed no signs of breeding coloration and were thus regarded as not reproductively active (Borg et al. 2004). During development (10 weeks post-hatching) and at adult stage fish were fed with defrosted red *Chironomus* larvae in excess every morning. All aquaria were cleaned daily from remaining mosquito larvae and excrements and replenished with fresh tap water.

To sum up, all fish used in this study experienced the same rearing conditions with respect to diet, lighting conditions and chemical water properties throughout their complete development, irrespective of their parents' habitat type.

### Experimental design

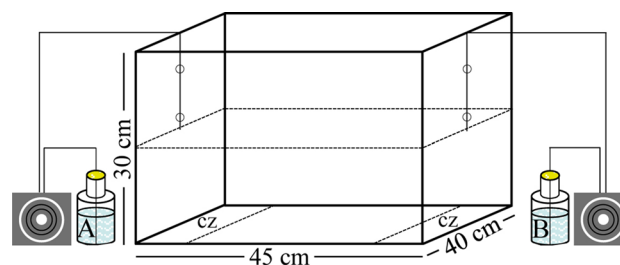
To test whether individuals of the different populations and habitat types were able to distinguish between their own population and a foreign population by odor alone four different trial types (see Table 1) were run using stimulus water from the own population versus stimulus water of different populations from the same or the different habitat (either tea-stained or clear-water). Overall 47 experiments were conducted in random order to avoid sequence effects.

Two days before an experiment started, three stimulus fish of one family and three stimulus fish of another family

as well as a test fish, which never originated from the same family as any of the stimulus fish, were randomly chosen and placed in three separate holding aquaria (30 cm × 20 cm × 20 cm). Shortly before, standard length (SL) and mass (M) were measured and the condition factor (CF) [ $100 * M \text{ (g)}/SL \text{ (cm)}^3$ ] following Bolger and Connolly (1989) was calculated. The aquarium containing the test fish was filled up to a water level of 13.5 cm, and each of two aquaria containing the three stimulus fish was filled with 1.53 liter per gram fish to keep the fish mass in relation to water amount constant. To avoid any influence of air-borne odors (McLennan 2004), the two stimulus tanks were always covered with transparent plastic sheets. All fish were fed once daily to excess with frozen mosquito larvae. Remaining larvae were removed from the aquaria 30 min after feeding using a flexible tube and the water thus extracted by suction was refilled into the tank to keep the amount of water constant.

The dichotomous choice experiment was conducted in a test glass aquarium (45 cm × 40 cm × 30 cm; Fig. 1) placed on a table and surrounded by a black curtain to prevent disturbances. Two 7.5 cm wide preference zones on both sides of the aquarium were marked by black solid lines drawn onto the bottom of the tank. The test fish behavior was recorded by camera (Logitech Carl Zeiss) attached centrally above the aquarium. Light was provided by a fluorescent tube (TrueLight, Natural Daylight T8/36 W) installed 59.5 cm above the aquarium.

Before an experiment, the test aquarium was filled with 1-day-old tap water up to a level of 15 cm. Thereafter, 300 ml of stimulus water was taken from each of the two



**Fig. 1** Test tank used for odor-preference tests. The figure shows the test tank with two 7.5 cm measuring choice zones (cz) on each side of the tank and two bottles (A and B) with 300 ml of stimulus water connected to two peristaltic pumps that transported the stimulus water simultaneously into the test tank via flexible tubes

**Table 1** Description of the four trial types, which were conducted in random order to avoid sequence effects

Trial type	$N$	Test fish	Stimulus 1	Stimulus 2
1	11	Tea-stained	Tea-stained (own population)	Tea-stained (other population)
2	9	Tea-stained	Tea-stained (own population)	Clear-water
3	13	Clear-water	Clear-water (own population)	Clear-water (other population)
4	14	Clear-water	Clear-water (own population)	Tea-stained

stimulus aquaria and filled into two small bottles. The bottles were connected to a peristaltic pump (4 ml/min) installed outside the curtain surrounding the setup on separate tables to avoid any influence of vibrations produced by the pumps (Fig. 1). The pumps were used to add the stimulus water via two flexible tubes, attached on the right and left side of the test aquarium (Fig. 1). The plastic tubes were attached in such a way that they discharged just slightly underneath the water surface (see also Mehliis et al. 2008, 2009). The sides of the different stimulus water samples were chosen randomly between experiments to control for side preferences of the test fish.

The test fish was placed in the middle of the test aquarium with a dip net and allowed to acclimate in neutral water for 15 min. Ten min after introduction, the camera recording was started, and after 14 min and 40 s, the peristaltic pumps were activated, so that 5 min after the start of the recording the water conditioned with the odor arrived in the test aquarium. The test fish was then observed via the live recording on a laptop until it had entered both preference zones once. That was to ensure that the test fish was aware of both olfactory stimuli (see also Mehliis et al. 2008, 2009). Once it had entered both choice zones (median; 1st quartile; 3rd quartile: 102 s; 43 s; 163 s), the total time the fish spent in the choice zones was recorded for 10 min. The observer was naïve with respect to the side where “own population odor” or “foreign population odor” was being introduced into the tank as well as naïve with respect to trial type. After each experiment, all objects used were rinsed with a solution of 3 % hydrogen peroxide in order to remove any residues of odor (McLennan 2004; Mehliis et al. 2008, 2009). All test and stimulus fish were only used once.

### Statistical analysis

The R 2.9.1 statistical package was used for all analyses (R-Development-Core-Team 2009). Linear mixed-effect models (“lmes”) were fitted using the “lme” function in the “nlme” library as data did not significantly deviate from normal distribution according to Shapiro–Wilk tests.

To check for a general preference of test fish for own population odor in comparison with foreign population odor, a preference index ( $\text{pref} = [\text{time (own population odor)} - \text{time (foreign population odor)}] / \text{total time in preference zones}$ ) was calculated and used as dependent variable in two models (clear-water and tea-stained). SL, CF and trial type (odor of own habitat type or odor of foreign habitat type) of the test fish were included as explanatory variables and the population and family the test fish originated from were included as random factors in all models. Four additional linear mixed-effect models were conducted to investigate preferences within the four

different trial types. In order to test for an influence of habitat type (tea-stained or clear-water), the preference index was used as dependent variable and habitat type was included as explanatory variable.

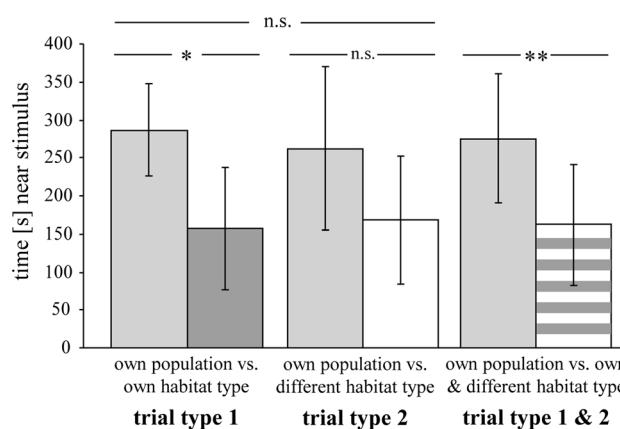
In all models, random factors were never removed to control for family- and population-specific odor preferences, respectively. Non-significant explanatory variables were stepwise removed from the models in descending order of significance. Test of significance was based on likelihood-ratio tests (LRTs). In addition, we used Akaike’s information criterion (AIC) for model comparisons; here, the model with the lowest AIC value represents the best-explaining model (Symonds and Moussalli 2011). The AICc, AIC values with a correction for infinite sample sizes, was also included.

### Statement of animal rights

The study conforms to the Association for the Study of Animal Behaviour guidelines for the use of animals in research as well as to the legal requirements of Germany and was carried out according to the German laws for animal experiments.

## Results

Fish from the tea-stained habitats significantly preferred the odor of water conditioned by members of their own population over that of a foreign population independent of habitat type (“lme”,  $N = 20$ ,  $t = 3.32$ ,  $P = 0.010$ ; Fig. 2). Standard length (SL) and CF and trial type were never included in the best-explaining model, respectively, did not



**Fig. 2** Time test fish from the tea-stained habitat spent near stimulus water conditioned by fish from their own population (light gray bars) versus a different population of the tea-stained habitat type (dark gray bar, trial type 1) or the clear-water habitat type (white bar, trial type 2) or the mean of trial type 1 and 2 (gray-white stripes). Data plotted are means  $\pm$  SE. n.s.  $P > 0.10$ , \* $P < 0.05$ , \*\* $P < 0.01$

**Table 2** Summary of the linear mixed-effect models for test fish from tea-stained and clear-water lakes (A), as well as for differences between habitat types (B)

Dependent variable	Explanatory variable	AIC	AICc	Explanatory variable	$\chi^2$	df	P
A							
Preference index of tea-stained	Trial type + SL + CF	25.269	26.769	Trial type	0.986	1	0.321
	SL + CF	24.254	24.960	CF	0.897	1	0.344
	CF	23.152	23.374	SL	1.021	1	0.312
	1	22.172	22.172				
Preference index of clear-water	Trial type + SL + CF	28.881	29.924	CF	0.043	1	0.835
	Trial type + SL	26.924	27.424	SL	1.247	1	0.264
	Trial type	26.172	26.332	Trial type	4.352	1	<b>0.037</b>
	1	28.524	28.524				
B							
Preference index	Habitat type	45.997	46.086	Habitat type	3.998	1	<b>0.046</b>
	1	47.995	47.995				

The dependent variable was the preference index [(time (s) near own population odor—time (s) near foreign population odor)/total time in preference zones]. Explanatory variables included were “SL” [standard length (cm)], “CF” (condition factor) and “trial type” (own habitat-type odor/foreign habitat-type odor) (A) or “habitat type” (tea-stained or clear-water) (B). To control for possible influences of population and family both variables were included as random factors and were never removed from models. Given are AIC values (Akaike’s information criterion) and AICc values (the model with the lowest AIC represents the best approximating model) as well as results of stepwise removal of explanatory variables and respective statistics. The results show that AIC and *P* values are closely linked (see also Murtaugh 2014). Significant results (*P* < 0.05) are printed in bold

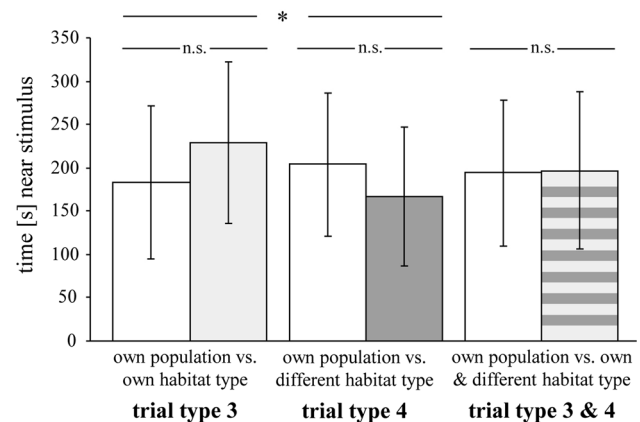
have a significant influence on observed preferences (Table 2A). When own population odor was tested against odor of the same habitat type, fish from tea-stained lakes showed a significant preference for own population odor (“lme”, *N* = 11, *t* = 2.81, *P* = 0.02; Fig. 2), while the preference for own population odor over odor of the different habitat type was not significant (“lme”, *N* = 9, *t* = 1.50, *P* = 0.18; Fig. 2).

Fish from the clear-water habitat did not show a significant preference for own population scented water over foreign population odor (“lme”, *N* = 27, *t* = 0.29, *P* = 0.78; Fig. 3). Standard length (SL) and CF were not included in the best-explaining model; however, trial type was included in the model and had a significant influence on observed preferences (Table 2A; Fig. 3). Test fish did not show a significant preference when own population odor was tested neither against own habitat type (“lme”, *N* = 13, *t* = -0.94, *P* = 0.37; Fig. 3) nor against foreign habitat type (“lme”, *N* = 14, *t* = 1.21, *P* = 0.25; Fig. 3).

The general preference of own population odor versus foreign population odor differed significantly for fish from both habitat types (Table 2B; Fig. 4).

## Discussion

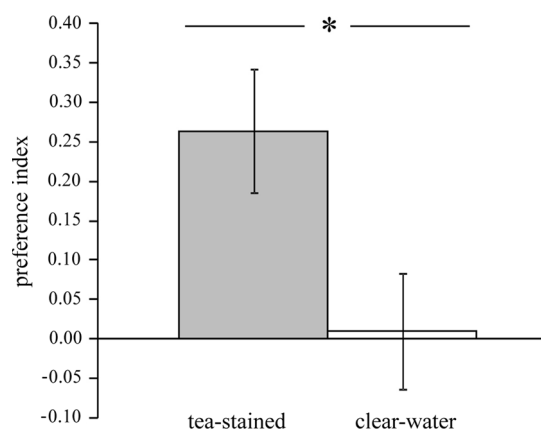
In accordance with our hypothesis, which postulated an increased use of the olfactory system and a better-developed olfactory communication in a habitat with limited



**Fig. 3** Time test fish from the clear-water habitat spent near stimulus water conditioned by fish from their own population (white bars) versus a different population of clear-water habitat type (light gray bar, trial type 3) or the tea-stained habitat type (dark gray bar, trial type 4) or the mean of trial type 3 and 4 (light-dark gray stripes). Data plotted are means  $\pm$  SE. n.s. *P* > 0.10, \**P* < 0.05

possibilities for visual communication due to turbid, tea-stained conditions (see Rauschecker and Kniepert 1994), we found a significant difference in the response to olfactory stimuli between sticklebacks derived from the two differing habitat types. Sticklebacks whose parents came from turbid, tea-stained lakes differentiated between own and foreign population odors, whereas sticklebacks whose parents came from the clear-water habitat showed no significant preference for own population odors. Testing the





**Fig. 4** Comparison of preference indices (time spent near stimulus water conditioned by fish from their own vs. a different population/total time spent in choice zones) between test fish from the tea-stained and clear-water habitats. Plotted are means  $\pm$  SE. \* $P < 0.05$

F1-generation of the different stickleback populations, which had all been reared under standardized conditions, suggests a genetic basis of the differential responses. However, even though the parental generation had been maintained in the laboratory 2 months prior to breeding, it cannot be ultimately excluded that odors were learned during the embryonic development. Nevertheless, effects of diet and recent habitat cues that have been shown to be important in sticklebacks' social decisions (e.g., Ward et al. 2004, 2005), should be of minor importance under our standardized conditions.

As mentioned above, recognition can be based on multiple sensory modalities and is dependent on the environmental conditions under which recognition occurs (Candolin 2003). A lack of recognition and/or differentiation on the part of sticklebacks from the clear-water habitat of course does not necessarily imply that they are not able to identify population-specific cues based on olfactory cues alone. The lack of differentiation might also be based on a lack of attraction to groups with conspecifics of the same population. However, the additional advantages of shoaling with familiars are multifarious (e.g., Chivers et al. 1995; Griffiths et al. 2004; Strodl and Schausberger 2013); thus, it is reasonable that the lack of differentiation is rather based on an inability to discriminate based on olfactory cues alone. This is supported by the result that sticklebacks from clear-water habitats differentiated significantly with respect to trial type: when tested against own habitat odor, they associated longer with the own habitat-type odor, while when tested against foreign habitat odor, they associated longer with own population odors. Thus, clear-water fish were obviously able to treat odors differentially. Other studies have shown that increased turbidity leads to an increased use of olfactory cues in guppies (Chapman et al. 2010) as well as in

sticklebacks concerning foraging (Webster et al. 2007) and mate-choice (Heuschele et al. 2009). A study on the populations used in the present study hinted at an increased use of visual cues by sticklebacks from clear-water populations in comparison with sticklebacks from tea-stained populations during courtship (Hiermes 2008). Here, stickleback males from clear-water habitats exhibited the stickleback typical red courtship coloration that has been shown to be decisive during courtship, mate-choice and male–male aggression (see Bakker and Milinski 1993), while sticklebacks from tea-stained habitats only had a dull courtship coloration but courted more vigorously, supposedly to compensate for the reduced visibility within their habitat of origin (Hiermes 2008). Furthermore, a study on a limnetic and benthic stickleback “species pair” has shown that wild-caught benthic females recognize males of their own species by odor, whereas limnetic females do not; this also corresponds to the ecological conditions and the habitat type of the “species pair” with benthics breeding in deeper water (Rafferty and Boughman 2006). Thus, it would be interesting to conduct further experiments offering visual cues or a combination of olfactory and visual cues and compare those again among sticklebacks of both habitat types and investigate whether the environmental lighting conditions indeed promote a differing use of sensory channels as suggested by the current study.

The results of the present study are in accordance with the results found by Ward et al. (2009). In detail, Ward et al. (2009) showed that wild-caught sticklebacks from two ecologically distinct populations (high water flow/high visibility vs. low water flow/low visibility) differed in the same way in their response to conspecific cues. However, whether recognition was based on extrinsic, e.g., habitat-specific (Behrmann-Godel et al. 2006) or diet-specific (Ward et al. 2004, 2005) or intrinsic cues remained unknown in this study (see Ward et al. 2009 for details). In our experiments, the F1-generation was used, which had been reared in family groups in the laboratory under identical conditions: the same turbidity conditions, the same chemical composition of the tank water (e.g., pH-value) and the same diet. During experiments, sticklebacks were never tested with family members (=fish from their own tank). Thus, it can be ruled out that recognition of population members was based on the confounding influence of kinship, familiarity and/or of tank-specific odors. Moreover, recognition on the basis of diet can also be excluded. Considering these facts it seems plausible that fish in the present study identified population members based on population-specific intrinsic odors that are expressed irrespective of extrinsic factors like diet and certain habitat-specific (in the field) or tank-specific (in the lab) odors.

A variable that has not been taken into account is that the two habitat types differ not only in water clarity but also in

pH. The tea-stained habitat on North Uist has a pH of about 6 and is characterized by a high amount of humic acids, while the clear-water habitat is more alkaline with a pH of around 8 (MacColl et al. 2013). A study on olfactory communication in sticklebacks has shown that an increase in pH (from 8.0 to 9.5) enhances the olfactory signal value of males in a mate-choice context, either by improved transmission of the chemical cues or by enhanced sensitivity towards the cues under these conditions (Heuschele and Candolin 2007). Further studies have also demonstrated a negative effect of low pH-values on olfactory communication in fishes (e.g., Moore 1994). Furthermore, humic acids, present in the tea-stained lakes, bind pheromones that are released into the water and leave them undetectable to chemoreceptors (Hubbard et al. 2002). Intraspecific communication based on olfactory cues in a swordtail fish (*Xiphophorus birchmanni*), for example, was disrupted in water with elevated levels of humic acids (Fisher et al. 2006). In our study, sticklebacks from tea-stained, low pH habitats were able to recognize members of their own population based on olfactory cues only, while sticklebacks from the clear-water habitat were not or were reluctant to do so. The use of olfactory cues despite the unfavorable chemical properties is likely an adaptation to the potentially even more adverse effects of turbidity on visual communication or might as well be the result of an enhanced sensitivity for olfactory signals precisely because chemical conditions for olfactory communication are inappropriate.

It is conceivable that the sensory systems and the use of different sensory cues in sticklebacks of both habitat types are optimally adapted to the respective environmental conditions. Experiments were conducted under standardized laboratory and pH conditions. It would be very interesting to test sticklebacks under the pH conditions of the habitats of origin, either under acidic and/or under alkaline conditions, and investigate whether the found differences might be even more pronounced under “natural” conditions or might fade under extremely different pH levels. To disentangle the influence of recent habitat experience, a split-clutch design would be interesting to conduct, rearing half of the fish under light and pH conditions of their ancestors and the other half under the opposite habitat conditions. Furthermore, it would be interesting to investigate the influence of other sensory cues, especially visual cues, and ponder the importance of these cues with respect to the varying environmental conditions.

To conclude, sticklebacks from two distinct habitats types varied with respect to their response to population-specific odor cues. The observed differences in odor preferences are likely the consequence of the, presumably genetically based, adaptation of olfactory sensory channel or a combination of sensory channels used in social recognition, which started with the retreat of the last Ice Age 10,000 years ago.

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**Conflict of interest** The authors declare that there are no conflicts of interest.

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