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Variations in sensitivity to chlorine in Ecuador and US consumers Implications for community water systems

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ABSTRACT

Successful implementation of chlorination for disinfecting community water systems in developing countries faces obstacles, with rejection of chlorinous flavor as a significant factor. Determining consumers' abilities to accurately detect chlorine in treated water is important to identifying acceptable chlorination levels that are also effective for water disinfection. Chlorine detection sensitivity was tested in untrained Ecuadorian consumers with limited prior experience with chlorinated water and US consumers with extensive prior experience with chlorinated water. Water samples with free chlorine concentrations up to 3.0 mg/L were presented for flavor testing. Ecuadorian consumers showed higher sensitivity, being able to detect chlorination at 2.0 and 3.0 mg/L, while US consumers did not reliably detect chlorine presence for any concentration levels. Additionally, Ecuadorian consumers' rejection of water samples depended on chlorination, showing a statistically significant increase in rejections of samples with chlorine concentrations above 1.0 mg/L. On the other hand, although US consumers rejected more samples overall, their tendency to reject did not vary as a function of chlorination levels. This study demonstrated that limited experience with chlorination is a critical factor for accurate chlorine flavor detection in drinking water.

Key words | chlorination, compromised acceptance, Ecuador, flavor perception, water treatment

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In 2012, an estimated 871,000 deaths worldwide were attributable to unsafe water, poor sanitation, and lack of hygiene practices (WHO 2016). Additionally, in 2015, an estimated 700 million people still used unimproved sources of drinking water, with a disproportionate number of those people residing in rural areas (WHO 2016). Improvements to clean water access, especially in rural areas, can have a significant positive impact on worldwide public health.

Chlorination is one of the most widely used interventions to treat drinking water for the removal and preclusion of biological contamination. Chlorine is a strong oxidizing agent, making it an excellent disinfectant (Deborde & von Gunten 2008). Chlorinating water has been shown to eliminate fecal indicator bacteria and *Escherichia coli* colonies, both of which can be signs of microbiological contamination (Quick *et al.* 1996; Luby *et al.* 2001). Home chlorination has also been effective at reducing diarrhea rates, suggesting removal of harmful water contaminants (Quick *et al.* 1997; Semenza *et al.* 1998; Mengistie *et al.* 2013). However, water treatment and even a reduction in indicator organism counts in home water samples are not always accompanied by decreased diarrhea rates (Kirchoff *et al.* 1985; Olembo *et al.* 2004). This could be due to poor hygiene habits (e.g. handwashing), as well as the fact that not all pathogenic species are marked by indicator organisms. Insufficient chlorine treatment may also result in the

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survival of viruses or pathogen-carrying protozoa which are more resistant to chlorine than are free-living bacteria (King et al. 1988). In addition, the persistence of diarrhea could be attributed to unsafe water storage practices or to family members drinking water from other untreated sources outside of their homes. Reliance upon the centralized treatment of community water systems could help prevent these latter causes of treatment failure. Chlorination is effective when it is maintained at concentrations sufficient to inactivate pathogenic microorganisms in the source water while also precluding their growth in distribution systems through the entire community water supply. It is important to note that water with high levels of natural organic matter (NOM) or turbidity requires a higher dose of chlorine for disinfection, and may require filtration prior to chlorine treatment to establish and maintain a chlorine residual for very high levels of NOM (Kotlarz et al. 2009). Chlorination, particularly when implemented with a community-wide (piped) distribution system, is a key contributor to public health improvements in underdeveloped areas.

While an effective treatment method, chlorination has its challenges – alternative approaches are broadly proved for systems that can provide safe drinking water without reliance on chlorine-based disinfection practices (Rosario-Ortiz et al. 2016). In addition to complexities involved in the physical operation of a community-wide water system with centralized chlorination, barriers to community acceptance also arise from concerns about price, accessibility, and flavor and smell of treated water, as well as knowledge and beliefs about water safety (Sperry & Billings 1921; Olembo et al. 2004; Luby et al. 2008; Freeman et al. 2009; Luoto et al. 2011). Among those, flavor is a central element of consumers' water evaluation, and changes perceived in water flavor that accompany chlorination can be a critical problem with acceptance of a water treatment system (Olembo et al. 2004; de França Doria et al. 2009; Freeman et al. 2009; Luoto et al. 2011). Chlorinous flavors are one of the most commonly cited objections to treated drinking water in the developed and developing world alike (Suffet et al. 1996; Firth et al. 2010; Luoto et al. 2011; Francis et al. 2015; Piriou et al. 2015). In the developed world, objectionable flavors might lead to a consumer opting for other safe sources such as bottled water rather than tap water (Doria 2006; Puget et al. 2010). However, in the developing world, the consequences can be more serious; if the flavors are unacceptable, a consumer will likely turn to an unsafe water source (Olembo et al. 2004; Luoto et al. 2011; Ritter et al. 2014). Improper management of chlorinous flavors in chlorinated water poses a public health threat by decreasing the chances of a community's acceptance of water treatment.

Because a change in water flavor can influence a community's response to chlorine-based water treatment interventions, community flavor perceptions ought to be studied before or alongside the implementation of a treatment system. Sensory tests for flavor have been used since the early 20th century to monitor the quality of treated water (Dietrich et al. 2004). As such, it is worth reviewing some of the tests available to researchers in this field, using the work of Dietrich et al. (2004) as a guide. There are two major categories of sensory tests: analytical and affective. Analytical tests require a controlled setting and trained consumer panels, while affective tests require less environmental control and analyst training. The requirements of these tests differ because the purposes of the tests differ.

The analytical tests focus on getting the most accurate measures of qualitative flavor descriptions and/or flavor intensities and can be further sub-categorized. Discriminative tests use panelists' ability to detect the presence or absence of a tastant or odorant as well as their ability to differentiate odors and flavors in order to understand sensory perceptions. These tests are either difference tests or sensitivity tests. Examples of different tests are paired comparisons, triangle test, duo trio test, and intensity ranking/rating tests, and examples of sensitivity tests include the constant stimulus test, ascending/descending triangle test, and method of limits. In addition to discriminative tests, descriptive tests use the most highly trained panelists to yield a sophisticated characterization of the flavor of a sample substance. Examples of these tests include various methods of relative attribute rating as well as flavor profile analysis and quantitative descriptive analysis. Many of these methods can be adapted to probe perceptions of flavors or odors. All of these analytical tests represent the best evidence-based methods to achieve an accurate description of a substance's (e.g. water) flavor.

Affective tests, on the other hand, differ from analytic tests in terms of purpose and implementation. These types of tests are primarily responsible for determining group preference for particular flavors over others or acceptance of a flavor according to a standard and involve large samples of untrained consumers as panelists. In the context of the present study, affective testing represents a way to explore water flavor perception by people who will consume the treated water when implementing a chlorination system in a developing global region. In all sensory tests, human subjects constitute the measuring instruments. It follows that training of panelists makes the instruments capable of more precise measurements, and vice versa. However, when faced with an imprecise instrument (i.e. an untrained consumer), we must control for error by taking many measurements and considering them all as a whole. This is exactly what happens with affective tests, as with the testing in our study: large samples of untrained consumers go through a sensory test procedure, and the whole of their measurements are summarized. In addition to flavor testing, additional methods have been developed for odor testing, including the Threshold Odor Number test and the rating method for evaluating distribution system odors in comparison to a control. However, the present study focused on flavor rather than odor testing and so these tests were not utilized in this study.

With all of the summarized testing options available, investigators must balance the tests' capabilities with the availability of resources, panelists, and laboratory space. In studies like the present one that occurs in rural, developing regions, many of the tests may be impractical to implement or may yield data not relevant to developing world applications. Dietrich *et al.* (2004) provide a summary of the demands of the sensory tests, with the 2-of-5 test, Sensonics Scratch & Sniff test, and a rating method for evaluating distribution system odors in comparison to a control test showing promise for use in resource-poor settings. In light of these options and insights, we aimed to implement a methodology that could provide measurements on the relevant dimensions to our purpose (understanding community acceptance of chlorine-treated water) and can be achieved with minimal resources in a developing region.

A testing methodology that potentially meets these requirements has been developed by Lima Filho *et al.* (2015) and was the basis for the methodology used in this study. This technique, rather than indicating a threshold to detect particular flavors, instead measures the concentration level of a substance in water at which its presence significantly decreases the proportion of people who accept the use of the water. This level is a concentration range known as the compromised acceptance threshold (CAT). However, if the concentration level is too high, people will reject that water source, a level known as the rejection threshold (RT), also expressed as a range. These thresholds (i.e. CAT and RT) are applicable to the case of water treatment with chlorine because many people are able to detect chlorine at levels necessary for disinfection (Piriou *et al.* 2015). They would allow for identifying chlorine levels that effectively treat water and are accepted by consumers, even if chlorine flavor is detected. It is important to note that the maximum safe concentrations of chlorine in water, 5 and 4 ppm recommended by the WHO (2017) and US EPA (2009), respectively, are well above the levels at which flavor-based rejection usually occurs in a community.

Adapting Lima Filho *et al.*'s (2015) sensory testing methodology, we performed a cross-national comparison of flavor preferences among people from communities in the mountains of Ecuador and from a city in the USA. The study location in Ecuador allowed partnership with local and international non-government organizations, as well as the local government, in order to do work in an area where water disinfection is being newly introduced. This meant that the work was immediately relevant for the local partners. The context also made it possible to do research with participants who were familiar with chlorine (in bleach form), but who had limited experience with chlorine-based water treatment. In addition, this setting offered an opportunity to inform and guide continued efforts toward improving community water supplies.

Our cross-national comparison follows Piriou *et al.*'s (2015) comparison of chlorine flavor preferences between Spanish and French consumers, in which detection thresholds differed according to the consumers' country of residence. Piriou *et al.* (2015) suggested that the differing water chlorination practices of each country shaped the chlorine flavor preferences of its citizens: consumers appear to become habituated to the level of chlorine delivered at the tap. French consumers, whose water is typically delivered with a lower chlorine concentration ($\leq$0.3 vs. $\leq$0.5–0.7 mg/L in Spain), had a lower detection threshold for chlorine flavor. In our study, the Ecuadorian participants had very little experience with chlorinated tap water, relative to US participants. Following findings of Piriou *et al.*, we hypothesized that the Ecuadorian participants, having had less prior experience with chlorinated water, would be more sensitive to chlorinous flavors and thus would detect chlorine at lower concentrations than would participants from the USA.

In addition, we sought to apply methodology that would still allow for the determination of a CAT for chlorine concentration, helping to specify ranges for effectively chlorinating water without leading to rejection of chlorinated water. WHO (2017) recommends a minimum of 0.2 mg/L chlorine residual at the tap under normal, non-emergency circumstances. Our maximum tested chlorine level (3.0 mg/L) was below US EPA (2009) and WHO (2016) maximum guidelines of 4.0 and 5.0 mg/L, respectively, which are based on public health concerns to maintain water safety, not flavor and odor considerations. Also, by limiting the maximum tested chlorine level to avoid offensive flavor conditions, participants were not exposed to levels that might lead to bias against future implementation of system-wide chlorine-based water treatment (due to a negative experience with chlorine in this study). This method allows for recommendations to be made regarding implementation and management of chlorine water treatment systems in rural communities.

MATERIALS AND METHODS

Methods varied to some degree in Ecuador vs. in the USA due to differences in language and materials used. In the following sections, subcomponents of the method are described first for Ecuador, then for the USA.

Participants

Ecuador participants

Participants in Ecuador were volunteers from five communities in the Cantones of Guamote and Colta. Given the geographic area in which the study was performed, participants were predominantly of native Quechua ethnicity. Most participants were native Kichwa speakers, with Spanish as their second language. At each community, participants were gathered by asking a community leader to arrange for 30 individuals, 18 or older, who could participate in the flavor test. The community leader then recruited as many volunteers as were available and eligible to participate. In the five participant communities, a total of 123 volunteers were included, 61 females and 62 males (Castug: 30, Pomachaca: 15, Ocpote la Merced: 23, Achullay: 30, Sanancahuan: 25). One female participant failed to complete the testing. Community size was indicated for some communities by the number of households as follows: Castug: 80, Pomachaca: 350, Octpote la Merced: 75, Achullay, 98. No population data were obtained for Sanancahuan.

In four of the communities, participants were people who were available and lived near the testing site. In the remaining community, Pomachaca, testing occurred during market day, so few community members were available. Therefore, the participants from that community consisted of students and teachers at the community school. The ages represented in our participants ranged from 18 to 85 years old ($M = 52.2$, $SD = 19.1$) and can be broken down in the following groups: 32 participants <40 years, 38 between 40 and 60 years, and 53 participants >60 years. Participants were not compensated.

US participants

Participants in the study in the USA consisted of 54 students, faculty, and staff of Calvin College in Grand Rapids, MI. The ages represented ranged from 19 to 69 years old ($M = 29.1$, $SD = 14.7$), with 21 females and 33 males. US participant ages can be broken down as follows: 43 participants <40 years, 13 between 40 and 60 years, and 3 participants >60. Participants came from a variety of ethnic backgrounds, and 48 (88%) had at least 1 year of experience drinking chlorinated city water as their primary water, with most far exceeding that amount of time. All had some prior experience with chlorinated water sources. Volunteers were recruited via email solicitation, and testing was carried out on three separate occasions to gather enough participants. Participants were compensated with lunch.

Materials and apparatus

Ecuador materials and apparatus

Water was obtained from each community's untreated water supply and was found to have no detectable free or total chlorine present. It should be noted that solutions were prepared with sodium hypochlorite, bleach, as the oxidant source; however, solution analysis with the Hach DR900 Multiparameter Portable Colorimeter reports units of mg/L of chlorine, consistent with the US EPA standard Method 4500 test for chlorine in drinking water. As a result, all solutions herein are reported in mg/L of chlorine rather than the prepared molarity of hypochlorite ion. Six different water samples were prepared for flavor testing with increasing free chlorine levels as follows: 0.0, 0.1, 0.3, 1.0, 2.0, and 3.0 mg/L. A 100-mL graduated cylinder, P1000 micropipet, and stock chlorinated solution (~200 mg/L free Cl_2) were used for serial dilution of the samples. The stock chlorine solution was obtained from the dosing tank of the community of Castug. After a 30-min residency delay, free and total chlorine levels of the samples were tested with a Hach DR900 using 25 mL DPD (N-N-diethyl-p-phenylenediamine) reagent powder pillows for color indication. The analysis of low chlorine concentrations, 0.02–2.0 mg/L, followed Hach Method 8021 for free chlorine and Method 8167 for total chlorine, while mid-range chlorine concentrations, 0.05–4.0 mg/L, followed Hach Method 10245 for free chlorine and Method 1025 for total chlorine concentrations. Free chlorine accounts for both hypochlorous acid and hypochlorite present in water. Total chlorine is the sum of free chlorine and chloroamines from ammonia and organic nitrogen. The prepared chlorine levels of flavor samples correspond to the free chlorine measurement. To validate that no measurable change in the free chlorine concentration occurred during tasting, the bottles were retested following completion of testing, yielding robust free chlorine concentrations. Additionally, time between water collection and flavor testing allowed all water to reach room temperature before testing. Samples were mixed in plastic condiment bottles marked to 750 mL. Tasting samples were given in 2-ounce plastic sauce cups. New cups were used for each sample to avoid cross-contamination.

Data were recorded by the investigators with pen and paper on survey printouts. The sheet reflected the original intention of having participants mark with a vertical line their liking of each flavor (left or right, as indicated by arrows) on a non-numerical linear scale anchored by a happy face and a frowny face, answer a question about whether either sample contained chlorine, and respond to a single yes or no question regarding rejection for each sample consumed (see Figure 1, depicting an English language version of the scale). However, due to difficulties with the Spanish language being a second language for both participants and investigators, the survey was conducted as a series of 'yes' or 'no' questions orally posed to participants in order to capture the desired data indicators. The rating scales were used only to indicate relative preference as communicated to investigators, rather than an absolute measure of liking each sample.

The survey and questions probed participants' ability to detect a difference between samples, choose a sample as the source of difference after detection, and give their attitude

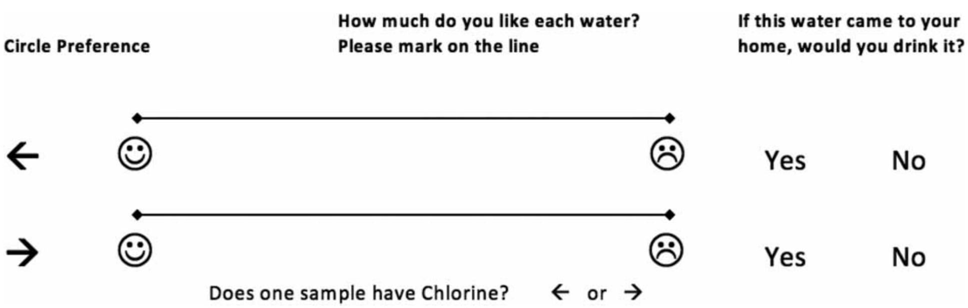

Figure 1 | Scales used for measuring liking, acceptability/rejection, and perception of chlorine flavor presence in pairs of water samples (arrow direction referred to the sample presented in the left or in the right of the participant's midline).

about the difference in flavors. The following series of yes/no questions, translated into Spanish, were asked for each pair of samples consumed:

1. Is there a difference in the flavors, or are they the same? (Detect)
2. If there is a difference, which flavor do you prefer? (Give attitude)
3. Does it seem that either of the samples contains chlorine? (Choose source)
4. If each water sample were the water that arrived at your home, would you drink it? (Give attitude)

A select few participants, particularly some of those more advanced in age, demonstrated significant difficulty in understanding and using the Spanish language. As such, volunteer interpreters were occasionally used to translate the questions and responses between the Spanish and Kichwa languages.

US materials and apparatus

Chlorinated water was prepared in a laboratory using nearly identical equipment to that which was used in Ecuador. All water used was Grand Rapids municipal water dispensed through taps in college academic buildings. Due to sparse use of many of the taps, the water had a long residence time in pipes, resulting in a tested free chlorine residual that was not detectable. On each testing day, an 8.25% sodium hypochlorite solution was diluted in tap water to make a stock solution. That stock solution was dosed into 500 or 800 mL of municipal tap water, depending on the requirements of each testing day. The same set of chlorine concentrations was used as those used in Ecuador, and free chlorine residuals were again verified with a Hach DR900 colorimeter. As in Ecuador, water samples were served at room temperature.

Questionnaires were similar to those used in Ecuador but presented in English (see Figure 1). Additionally, with the US sample, the liking scales were used as originally intended. Participants were instructed to draw a vertical line to mark their liking for each sample on the non-numerical continuum of 10 cm, anchored by a frowny face on the left and a smiley face on the right (Figure 1).

Detailed chemical analysis showed the US tap water to be highly similar to the water found in the Ecuadorian communities in which testing was done (see Table 1). The values for essential features including pH, total dissolved solids and electrical conductivity across samples were similar and satisfy tap water quality requirements across all tested localities (US EPA 2009). Nonetheless, the pH dependence of chlorine speciation and volatility, especially around the pK_a value of 7.6 for hypochlorous acid, may introduce differences in our tested water flavors. This potential bias is addressed in the Discussion. Flavor constituencies across US and Ecuadorian samples should also be highly similar. Species known to induce flavor (e.g. sulfate, sodium, and chloride) were measured and found to occur at levels below their respective flavor perception thresholds (Burlingame *et al.* 2007; Dietrich & Burlingame 2015). While some sodium levels in Ecuador did exceed flavor perception thresholds documented for young consumers, they still meet US EPA recommended standards and do not approach levels at which the flavor becomes objectionable or even widely recognized (Dietrich & Burlingame 2015). As such, the control waters were considered to

Table 1 | Comparison of chemical constituencies of US tap water and Ecuadorian water sources

	US Tap	Ecuador Average	Ecuador Maximum	Ecuador Minimum
Sodium (Na^+)	11.5	39.9	68.9	6.1
Potassium (K^+)	1.6	8.9	13.1	2.6
Chloride (Cl^-)	18.3	4.6	8.7	0.6
Nitrate (NO_3^-)	1.5	17.8	37.0	0.6
Sulfate (SO_4^{2-})	31.2	15.5	29.3	0.5
Bromide (Br^-)	0.6	0.3	0.5	0.0
Phosphate (PO_4^{3-})	1.6	1.0	1.4	0.6
Total hardness ($Ca^{2+} + Mg^{2+}$)	48.5	50.8	69.6	16.6
Magnesium (Mg^{2+})	12.1	23.1	36.2	4.6
Calcium (Ca^{2+})	36.4	27.8	39.4	12.0
pH	7.6	8.1	8.6	7.4
Electrical conductivity (μS)	280	440	834	107
Total dissolved solids	138	232	416	54

Note: All units are ppm unless otherwise stated.

have a highly similar flavor composition across all testing locations.

Procedure

Ecuador procedure

Chlorine solutions for tasting were prepared for each community by using untreated water from that community's water source and dosing small amounts of concentrated stock sodium hypochlorite solution. The same source water was used in both Achullay and Sanancahuan due to time limitations, geographic proximity of the two communities, and chemical similarity between water sources.

Six total water tasting samples were prepared at the following free chlorine levels, with 95% confidence intervals reported (in parentheses): 0.0 mg/L, 0.1 (0.06–0.15) mg/L, 0.3 (0.29–0.37) mg/L, 1.0 (0.91–1.08) mg/L, 2.0 (1.82–2.14) mg/L, 3.0 (2.76–3.59) mg/L. Following the same procedure used in Ecuador, the 0.1–1.0 mg/L levels were verified on the DR900 low range program (#80) with a 25-mL liquid sample, while the 2.0–3.0 mg/L levels were verified on the mid-range program (#87) with a 10-mL liquid sample. Free and total chlorine levels of each sample were verified from the dosing bottles immediately before and after testing in each community over the course of the experiment.

As testing was carried out, one investigator poured the samples into 2-ounce plastic cups immediately before serving, always pairing a blank control sample with a sample of each level of chlorination, in ascending order of chlorination. That investigator then placed the cups, uncovered, in front of the participant, indicating only to the data-recording investigator which sample was chlorinated. Each participant began by tasting a pair of blank controls, after which the concentration in one of the sample cups of every pair was increased with each tasting, from 0.1 to 3.0 mg/L.

When the chlorine solutions were prepped, participants were divided into groups of 10–15. Groups of these sizes were selected to allow enough wait time between tastings for residual flavor to fade, without causing an excess amount of waiting time for participants. After participants were gathered, instructions were given and each participant was assigned a number indicated on a small card handed to the individuals. Regarding the chlorination levels, participants were told only that all levels were safe, and that some tasting samples contained chlorine while others did not. From there, any distinguishing of chlorine flavor was the job of the individual participants. Verbal consent was obtained at the beginning of each testing session. A classroom, church hall, or open room was used for each implementation of the study, and participants sat around the room with the investigators at the front.

When an individual's number was called, that participant came forward to taste a pair of water samples. At the time of the first tasting, the recording investigator first documented the individual's age. Each time a participant came forward, the investigator indicated to the participant which sample of the pair to drink first. Drinking sequence, as well as spatial location (left–right) of the chlorinated samples, was randomized to control for order effects. Upon a participant's drinking of the samples, the investigator asked the series of yes/no questions, recorded responses, and asked the participant to be seated again. Participants of each group cycled through at each level of chlorination, allowing at least 2 min for residual flavor sensation to fade between trials. When every participant in each group had tasted all samples, number cards were collected, and participants were thanked for their participation and dismissed. Finally, water in the sample bottles was tested to ensure free chlorine level maintenance.

US procedure

The water was chlorinated and samples poured prior to participants gathering for testing. Actual free chlorine levels were measured at the beginning of each testing session. Target values remained the same as those used in Ecuador, and the actual measured values for each of the three testing sessions are reported here in parentheses: 0.0 mg/L, 0.1 (0.10, 0.08, 0.15 mg/L), 0.3 (0.29, 0.28, 0.32 mg/L), 1.0 (1.00, 1.03, 1.04) mg/L, 2.0 (2.01, 2.06, 2.09) mg/L, and 3.0 (3.07, 3.15, 3.08) mg/L. Poured samples remained uncovered for a short time (5–20 min) prior to tasting. Upon arrival in the classroom in which testing occurred, participants completed a consent form and water history questionnaire detailing the water that each participant was accustomed to drinking. They then received instructions on how to properly complete the questionnaire provided to them for data recording. Participants came to the front of the room, in order, to collect each pair of water samples. The participants then brought the samples back to their desks to taste and rate the waters. In the USA, data were recorded on pen and paper handouts by the participants themselves. Given the greater ease of communication in English, the oral questioning method used in Ecuador was deemed unnecessary. This allowed for more rapid testing, with groups of up to 22 participants at a time.

As in Ecuador, one of the samples in each pair was always blank, while the other sample ascended in free chlorine concentration from the initial blank of 0 mg/L up to 3.0 mg/L. At least 2 min was allowed between tasting of each sample pair to minimize interference of flavors from previously tasted samples (Piriou *et al.* 2015). Participants were instructed to alternate drinking the sample to their left or right first, while the chlorinated sample was randomly presented on either of the participants' left- or right-hand sides. Upon tasting all the samples, all materials were collected and data were coded. Liking scales were coded by measuring the distance from the inside of the smiley face to the participants' mark, in centimeters, such that a larger distance represented a more disliked flavor.

RESULTS

The data were analyzed to address the question of whether Ecuadorian and US groups differ in their ability to detect chlorine in water and the rate at which they reject water samples that varied in chlorination levels. Comparing these rates across groups would allow us to address the hypotheses that past experience with chlorination impacts flavor perception and acceptance of chlorinated water.

First, in order to determine whether groups differed in their abilities to correctly identify samples that contained free chlorine, separate two-way chi-square analyses were performed on the data from the Ecuador and the US participants. Counts of participants that correctly indicated which samples contained free chlorine (identified a chlorinated water sample as containing chlorine) were compared to those that incorrectly identified a non-chlorinated water sample as containing chlorine for each of the levels of chlorination (0.1, 0.3, 1.0, 2.0, and 3.0 mg/L) to determine the threshold at which participants could correctly identify chlorinated water. Participants who indicated that neither sample in a pair contained chlorine were not included in this analysis. The analysis of the Ecuador participants showed a significant effect, X^2 (4, 270) = 17.899, $p = 0.001$. See Figure 2 for the counts involved in this analysis. Following this significant effect, pair-wise chi-square analyses were conducted on the number of participants who gave correct indications of chlorine presence vs. the number who gave incorrect indications of chlorine for each of the five levels of chlorination. The following results were found: $X^2_{0.1}$ (1, 35) = 0.257, $p = 0.612$, $X^2_{0.3}$ (1, 42) = 3.429, $p = 0.064$, $X^2_{1.0}$ (1, 54) = 1.185, $p = 0.276$, $X^2_{2.0}$ (1, 69) = 32.014, $p < 0.001$, $X^2_{3.0}$ (1, 70) = 22.857, $p < 0.001$. The number of correct identifications was significantly higher than the number of incorrect indications when free chlorine surpassed the 1.0-mg/L level. Analysis of the data from the US participants indicated that participants did not successfully differentiate chlorinated samples from unchlorinated samples as a function of chlorination level as there was not a significant difference between the number of correct identifications vs. the number of incorrect identifications, X^2(4, 203) = 4.093, $p = 0.394$. See Figure 3 for the counts involved in this analysis. Taken together, the outcomes of these analyses indicate that the participants from Ecuador successfully distinguish chlorinated from non-chlorinated samples above 1.0 mg/L, while the US participants did not

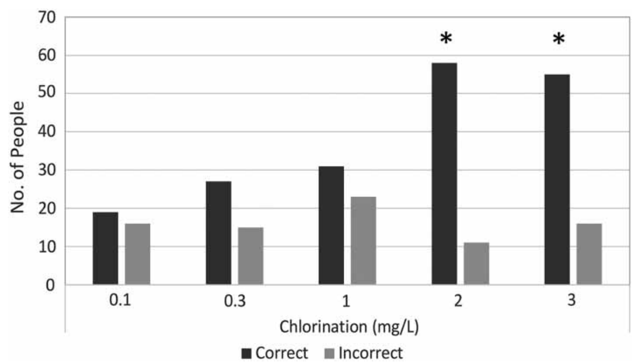

Figure 2 | Counts of Ecuadorian participants (of $N = 123$) who correctly identified chlorinated water samples as chlorinated vs. counts of those who incorrectly identified non-chlorinated water samples as chlorinated for each level of free chlorination. * = significant X^2, $p < 0.05$.

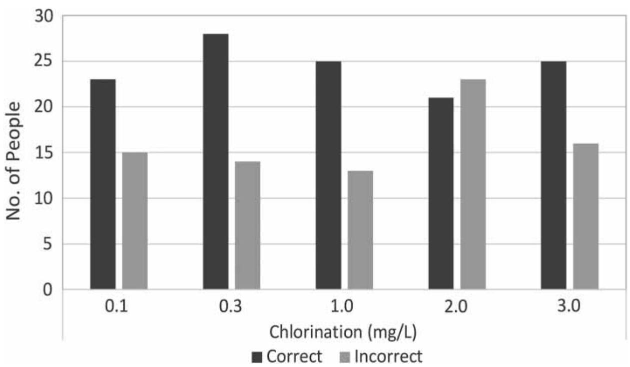

Figure 3 | Counts of US participants (of $N = 54$) who correctly identified chlorinated water samples as chlorinated vs. counts of those who incorrectly identified non-chlorinated water samples as chlorinated for each level of free chlorination. No significant effect.

show such sensitivitiy, being unable to differentiate chlorinated from unchlorinated samples across levels of chlorination.

Second, chi-square analyses were also performed to assess chlorine concentration levels at which participants begin to reject water samples, relative to samples without chlorine. A two-way chi-square analysis could not be conducted to compare a number of rejections of chlorinated vs. non-chlorinated waters because some participants rejected both the chlorinated and non-chlorinated waters for some sample pairs, and thus some participants would belong to both conditions. This situation violates the independence assumption of chi-square analyses. Therefore, one-way chi-square analyses were conducted only on participants who rejected the chlorinated water sample at each of the five levels of chlorination. The result of the one-way chi-square for the Ecuador participants was significant, X^2 (4, 65) $= 13.231$, $p = 0.010$. See Figure 4 for the counts involved in this analysis. Following this significant effect, pair-wise chi-square analyses were conducted to compare the number of Ecuadorian participants who rejected the chlorinated sample at each of the five levels of chlorination compared to the number of rejections at the 0.0 mg/L level, using the 0.0 mg/L level as a baseline for how many people rejected the water when chlorine could have no effect. The following results were found: $X^2_{0\text{–}0.1}$ (1, 12) $= 0.000$, $p = 1.000$; $X^2_{0\text{–}0.3}$ (1, 15) $= 0.600$, $p = 0.439$; $X^2_{0\text{–}1.0}$ (1, 16) $= 1.000$, $p = 0.317$; $X^2_{0\text{–}2.0}$ (1, 26) $= 7.538$, $p = 0.006$; $X^2_{0\text{–}3.0}$ (1, 26) $= 7.538$, $p = 0.006$. These results indicate that only when chlorine concentration passed the level of 1.0 mg/L, did the rejections of the chlorinated water significantly exceed the baseline number of rejections that occurred when no chlorine was present. The result of the one-way chi-square of the US participants was not significant, X^2 (4, 59) $= 0.576$, $p = 0.966$ (see Figure 5), indicating that for these participants, rejection rates did not differ as a function of chlorination levels.

Critical to determining the CAT is the ability to relate how much people like the water samples to rates of rejection. Liking scores were not obtained from the Ecuadorian participants, so CAT could not be calculated for that group. However, it was possible to attempt to calculate CAT for the US participants. Responses to the liking scale were measured for each participant for each paired sample type (chlorinated and non-chlorinated) for each

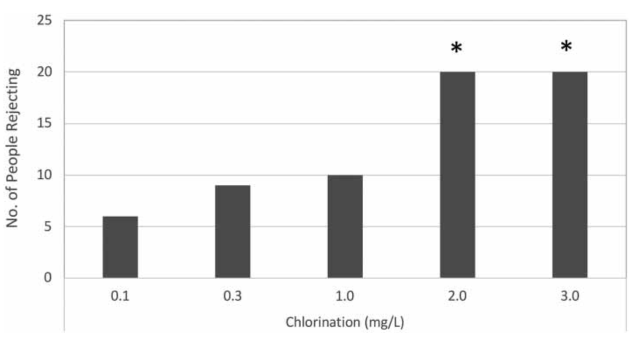

Figure 4 | Counts of Ecuadorian participants (of $N = 123$) who rejected chlorinated samples of water as a function of free chlorination level (mg/L). * = significant X^2, $p < 0.05$, for the number of participants rejecting at a level compared to the number of participants rejecting water with no chlorine.

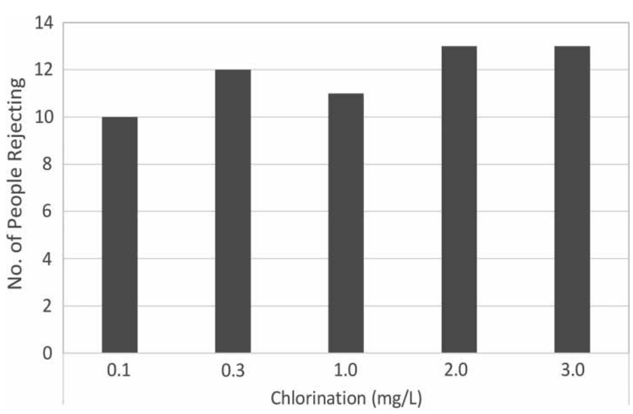

Figure 5 | Counts of US participants (of $N = 54$) who rejected chlorinated samples of water as a function of free chlorination level (mg/L). No differences are significant.

level of chlorination (0.1, 0.3, 1.0, 2.0, and 3.0 mg/L). To determine whether mean liking differed as a function of the independent variables' sample type and level of chlorination, a two-way, repeated-measures analysis of variance was conducted (see Figure 6 for means involved in this analysis). Data from one participant were excluded from this analysis because that individual failed to complete the scales, leaving us with $N = 53$. Neither the main effect for sample type, F (1, 52) = 1.186, $p = 0.281$, $\eta_p^2 = 0.022$, nor the main effect of level of chlorination, F (4, 208) = 0.461, $p = 0.764$, $\eta_p^2 = 0.009$, was significant. In addition, the sample type by the level of chlorination interaction did not reach significance, F (4, 208) = 1.694, $p = 0.153$, $\eta_p^2 = 0.032$. These results indicate that liking scale ratings did not vary as a function of characteristics of the water sampled. Combined with the finding that the rejection of water samples did not vary as a function of chlorine concentration levels for US consumers, CAT could not be calculated for the US participants.

Finally, the total number of participants who accepted and rejected samples was compared between the two countries to determine if there was a cross-national difference in water rejection, regardless of chlorine presence. Of the 540 water samples tasted in the USA, 438 (81.1%) were accepted, while 102 (18.9%) were rejected. Of 1,230 water samples tasted in Ecuador, 1,137 (92.4%) were accepted and 93 (7.6%) were rejected. A two-way chi-square analysis was conducted on these values, $X^2(1, 1,770) = 49.120$, $p < 0.001$. These results indicate that as a whole, and regardless of chlorine presence or absence, US participants were significantly more likely to report the water flavor to be unfit for drinking.

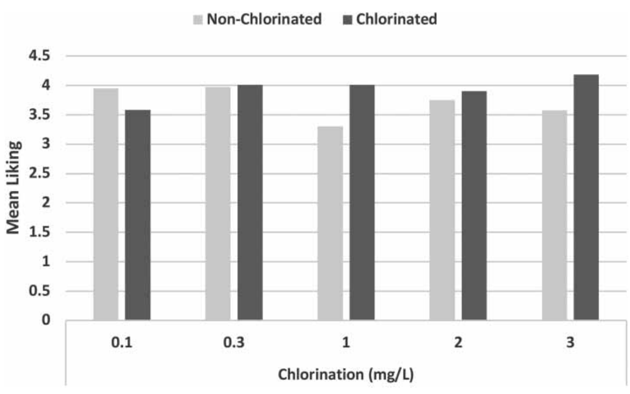

Figure 6 | Mean liking of chlorinated vs. non-chlorinated samples for US participants ($N = 53$) as a function of free chlorination levels. No effects are significant.

DISCUSSION

We performed affective testing of chlorine flavor perceptions with large untrained consumer panels in rural Ecuador and suburban Michigan, USA. The results support the hypothesis that individuals in rural Ecuador tend to be more sensitive to the flavor of chlorine than are US participants. Interestingly, US participants rejected a higher percentage of water samples, compared to Ecuadorians. However, the data from US consumers suggest that this effect was not due to consumers' sensitivity to the presence of chlorine impacting their flavor preferences, as they were not able to reliably differentiate chlorinated from non-chlorinated samples. Rather, US consumers simply had stronger preferences regarding water flavor, while Ecuadorians demonstrated greater sensitivity to chlorinous flavor than did US consumers.

One possible explanation for this high rate of rejection among the US participants is the adaptation of US consumers to water choices. Whereas the Ecuadorian participants typically drink water from only one source, the US participants have used water from various sources and have the freedom to select drinking water for preferred flavors. Consumers in the USA and developing regions have been shown to make decisions about their water based on a number of subjective judgments about factors like health impacts, organoleptics, and costs, and US consumers have sufficient options to develop a selective preference (Güngör-Demirci *et al.* 2016; Jeuland *et al.* 2016). The greater appearance of rejection across all chlorine levels among US consumers may represent greater selectivity for more general water flavor, rather than selectivity against chlorine specifically.

The observation of increased detection and rejection above the 1.0-mg/L level is a finding supported by previous work. Standing alone, our findings would appear to support the common target dose (following WHO guidelines for disinfection) of 2 mg/L as an effective maximum level for both disinfection and limiting chlorine flavor problems.

While previous work would agree that chlorine flavor detection and acceptability thresholds are likely to occur above 1 mg/L, those effects have been found to arise closer to 1 than 2 mg/L (Lantagne 2008; Jeuland et al. 2016; Crider et al. 2018). Therefore, 2.0 mg/L free chlorine could represent a maximum flavor acceptability standard for some settings (e.g. Ecuador). This standard cannot perfectly generalize, however, due to geographic and cultural differences in chlorine flavor perceptions, seen most clearly in the fact that French and Ethiopian consumers have been found to detect chlorine residuals at 0.14 and 2.0 mg/L, respectively (Lantagne 2008; Puget et al. 2010). Overall, a balance of the findings suggests that dosing practices ought to aim for maximum chlorine residuals between 1.0 and 2.0 mg/L, but favoring levels well below 2.0 mg/L in order to minimize chlorine flavor concerns. Such a reduction in target dose is also supported by previous work that has found chlorine dose reduction to limit bad flavors while maintaining microbiological water quality (Chiller et al. 2004).

In spite of being less selective about flavor in general, Ecuadorian participants demonstrated a greater sensitivity to chlorine specifically. As chlorine concentration rose, so did the Ecuadorians' rejections and correct identifications of chlorine flavor. Such a trend was not found among US participants. Given the water histories of each set of participants, a habituation explanation is likely, because we are generally more sensitive to stimuli with which we are not familiar. Given that those in the USA often drink chlorinated water much of their lives, while the Ecuadorian participants were mostly new to chlorinous flavors, it is reasonable that the Ecuadorian participants should be more sensitive to the new flavor. This finding is also consistent with our expectation from previous work that water preferences would vary according to participants' water treatment history (Piriou et al. 2004, 2015). It is important to note that previous work has found that US consumers detect chlorine at 0.8 or 1.1 ± 0.6 mg/L, differing from our US consumers' lack of sensitivity to chlorine flavor (Mackey et al. 2004; Piriou et al. 2004). However, great variability in individual thresholds preserves the possibility of this study's sample being less sensitive to chlorine. In all, a discrepancy in the absolute value of US detection thresholds does not take away from the conclusion that the relative flavor perceptions found in this study agree with past literature that one's history with chlorine water treatment matters for chlorine flavor perceptions; less previous exposure predicts greater sensitivity.

One of the limitations of the present study is that CAT (Lima Filho et al. 2015) could not be calculated for either the Ecuadorian or US groups. Future studies would benefit from careful collection of liking scale data and perhaps a higher range of chlorination than the 3.0 mg/L level tested in this study in order to enable the determination of a CAT.

In addition to limitations, there are a few sources of intergroup bias that ought to be addressed, namely participant age, water temperature, and pH. First, Ecuadorian participants were significantly older than those in the USA. Age impacts on odor sensation, suggesting that this would make the Ecuadorian participants less sensitive to chlorine odors than the US participants (Doets & Kremer 2016), yet we found Ecuadorians to be more sensitive. Similarly, the cooler climate (and also room temperature) in Ecuador, relative to the USA, should yield lower chlorine volatility and odor in the Ecuadorian water samples. This would make chlorine detection more difficult for Ecuadorian participants. Finally, Ecuadorian and US water samples differed somewhat in pH. Hypochlorous acid has a pK_a value of 7.53, such that increasing pH above that level will favor the less volatile hypochlorite ion, while more acidic pH levels (below the pK_a value) favor the more volatile hypochlorous acid species. When comparing water analyses in Table 1, most Ecuador communities had pH above that measured in the US water samples. This would suggest a greater prevalence of hypochlorite ions in the Ecuador water and therefore a reduced odor to be detected by participants. All three sources of bias suggest that Ecuadorian consumers would have a more difficult time detecting and identifying chlorinous odors. Our results, however, show the opposite pattern with Ecuadorian participants being more sensitive to chlorine flavor. Thus, Ecuadorian's ability to detect and identify chlorine flavor in spite of these biases strengthens our findings.

The findings of this study have important implications for water treatment implementation in developing regions. Namely, chlorine flavor perceptions yield biased consumer reports that put a downward pressure on chlorination efforts, impeding community acceptance of a treatment system. Even at the lowest chlorine levels, some participants rejected the water. However, those rejections were largely unwarranted or may be attributable to particularly sensitive individuals. Rejections and chlorine identifications at those low levels remained infrequent and not significantly different from the frequency of rejections for non-chlorinated water until chlorine concentrations were above 1.0 mg/L. Even at these higher levels of chlorination when significantly more participants rejected the water, only 16.3% of all consumers rejected chlorinated water. It is important to note that this high chlorination level is well above the Ecuadorian government's mandated minimum chlorine residual of 0.3 mg/L, as well as WHO minimum guidelines for effective chlorine residuals (Instituto Ecuatoriano de Normalización 2014; WHO 2017). As such, the maintenance of chlorine residuals at or below 1.0 mg/L appears capable of both effective disinfection and minimization of water rejection on the basis of flavor. Another noteworthy finding is that even when chlorine residuals are at their lowest effective levels, the water flavor will likely generate a small number of consumer complaints. Additionally, consumers may cite chlorine perception as the reason for complaints even in situations in which our findings suggest they would be equally likely to indiscriminately reject unchlorinated and chlorinated water. It follows that water treatment managers must be careful in how they respond to complaints from their consumers regarding chlorinous flavors. That is, before modifying treatment practices in response to consumer complaints, the presence of intolerable chlorine levels ought to be verified by testing with more objective instruments. Our findings give evidence that, without objective instrumental verification, biased consumer reporting may exert a negative influence on water disinfection efforts.

CONCLUSIONS

Flavor perceptions are highly subjective, making the formulation of broadly applicable standards difficult (AWWA Water Quality Division Taste and Odor Committee 2002). The treatment of water with other disinfectants (e.g. ozone) and vigilant distribution system monitoring are alternative approaches that can reduce or eliminate the flavor concerns of chlorine-based disinfection practices. However, chlorine treatment (with residual disinfectant) may be the preferred approach, especially when the water quality in distribution systems can be compromised by regrowth of pathogenic microbes and is insufficiently characterized by limited monitoring efforts. Providing information to water treatment managers and consumers regarding the flavor impacts of chlorine treatment is an important part of considering and implementing a treatment system in a new community.

With chlorine treatment, consumers ought to be continually included as instruments for measuring flavor impacts after the introduction of treatment in order to inform local dosing practices (Spackman & Burlingame 2018). Given that a small minority of people perceived flavor impacts even at low chlorine concentration levels, it is clear that flavor and odor impacts of chlorine disinfection of drinking water are unavoidable in some consumers. As such, it is incumbent upon the leaders of water treatment programs to understand consumer reports and use objective measures in combination with consumer reports to assess water quality and ensure that people are provided the highest quality and safest water possible. In addition, chlorine concentrations in community water systems need to be carefully controlled. Excessively chlorinated water that generates a negative public reaction (resulting in increased rejection) as well as ineffectively low chlorine levels that do not effectively treat water (resulting in a false perception of safety) would foster perceptions that undermine effective implementation of chlorine disinfection. Our findings of increased sensitivity to chlorine flavors for those with little treatment history suggest benefits of beginning chlorine water treatment at lower doses before raising the dosage as consumer flavor perception adapts to the new chlorine species. Ensuring adherence to new water treatment programs is difficult, and so beginning at lower doses can reduce the likelihood of such negative events that might further limit community uptake of treatment systems (Shaheed *et al.* 2018). Finally, influencing consumer perceptions that are deeply rooted in culture and experience may also be difficult. Achieving change in traditional water procurement and consumption practices in order to provide safe drinking water, while accounting for consumer perceptions of flavor, requires that water quality and chlorine levels be carefully controlled and that consumer reports be realistically evaluated in order to provide water that is consistently both palatable and potable.

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