INTRODUCTION

Vertebrate teeth display an enormous diversity in shape, size and structure. There is furthermore considerable variation concerning the location of teeth within the oral cavity, their number, their position on particular tooth-bearing bones, as well as in the sequence of tooth replacement (Huysseune & Sire, 1998; Stock, 2007). Several features of the dentition are assumed to be taxon-specific and are used as criteria by taxonomists for species identification and taxonomical classification (Cox & Hautier, 2015; Davalos, Velazco, Warsi, Smits, & Simmons, 2014; Huysseune, 2000). Despite the fact that basic processes in tooth formation are highly conserved among vertebrates (Huysseune & Sire, 1998; Underwood et al., 2015; Witten, Sire, & Huysseune, 2014), and that the development of the dentition is considered to be well canalized (Cox & Hautier, 2015), numerous studies have shown the occurrence of variations in dental patterns and in tooth shape (Roth, 1992; Golubtsov, Dzerjinskii, & Prokofiev, 2005; Mahler & Kearney, 2006; Shkil, Levin, Abdissa, & Smirnov, 2010; Cox & Hautier, 2015; among others). Alterations in the shape of the teeth, their number and the number of tooth rows are mostly assigned to one of the following categories: modifications as a symptom of pathological processes; acquired alterations, such as wear marks or traumatic tooth loss; and intraspecific variation (Cox & Hautier, 2015; Eastman & Underhill, 1973; Huysseune, 2000). Furthermore, alterations in tooth number and shape are part of the development during ontogeny (Boilotovskiy & Levin, 2011; Van der Heyden, Huysseune, & Sire, 2000; Van der Heyden, Wautier, & Huysseune, 2001).

Actinopterygians, which comprise more than half of the extant vertebrate species, are important models that are studied to
understand the mechanisms of vertebrate dental diversification (Stock, 2007). Within this group of ray-finned fishes, the family of Cyprinidae with over 3,000 species (Eschmeyer & Fricke, 2015), is one of the most speciose groups with an enormous diversity in tooth patterns (Pasco-Viel et al., 2010). Previous studies have described in detail regular and aberrant dental formulae for various cyprinid fishes.

To describe a dentition, the number of rows and number of teeth within a row are expressed as a formula (for the entire jaw) or as a half-formula (for one jaw half). Eastman and Underhill (1973) revealed widely differing degrees of intraspecific variation in the pharyngeal tooth formulae in Minnesota cyprinids. The percentage of branchial arches bearing variant half-formulae was species-dependent and ranged from 0% to 49.2%. In various species of cyprinids such as the spottail minnow (Notropis hudsonius), small African barb (Barbus paludinosus), snowtrouts (Schizothorax oconnori and Schizothorax raraensis) and the large African barb (Labeobarbus intermedius), the tooth formula is plastic. In these species the supernumerary teeth or additional rows of teeth are observed frequently (Eastman & Underhill, 1973; Golubtsov et al., 2005; Shkil et al., 2010). In contrast, the tooth formula appears to be static in the bluntnose minnows (Pimephales promelas, Pimephales notatus, Pimephales vigilax), speckled chub (Macrhybopsis aestivalis), gravel chub (Erimystax x-punctatus), and blacknose shiner (Notropis heterolepis) (Eastman & Underhill, 1973).

Heterochrony, a change in the developmental rate and timing (Hall, 1984), is assumed to have played an important role in diversification of morphology during evolution (Alberch & Alberch, 1981; Gould, 1977; Hall, 1984; McNamara, 2012; Raff & Kaufman, 1991). In particular, it has been proposed as one of the mechanisms possibly underlying alterations of skeletal characters in teleosts, such as restructuring, as well as loss and gain of elements (Kapitanova & Shkil, 2014a,b). To study the possible role of heterochrony in eliciting dental variability in cyprinids, we focused on the zebrafish (Danio rerio), a small polyphodont freshwater fish and nowadays an important model organism for studies on vertebrate development, genetics, and for biomedical research (Apschner, Schulte-Merker, & Witten, 2011; Bruneel & Witten, 2015; Van der Heyden et al., 2000). The dentition of wild-type zebrafish is well characterized and the establishment of tooth pattern, tooth succession and tooth development in zebrafish has been described extensively by Van der Heyden et al. (2000, 2001), and Huysseune and Witten (2006) for the different life stages.

In D. rerio, as in all other cyprinids, the dentition is restricted to the fifth ceratobranchial; the fish lack teeth on the oral jaws (Van der Heyden et al., 2001). The tooth formula in wild-type zebrafish appears to be invariant (Stock, 2007; Van der Heyden et al., 2001) and intraspecific variation in the tooth formula of wild-type zebrafish, to our knowledge, has not thus far been reported. The dentition is composed of 11 teeth distributed over three tooth rows on each ceratobranchial. These tooth rows extend rostro-caudally and contain five, four and two teeth in the ventral, mediodorsal and dorsal row, respectively (Figure 1). Replacement teeth arise ventrally and slightly posterior to their predecessor. Tooth development is divided into partly overlapping stages: the phase of initiation, of morphogenesis, of cytodifferentiation, and the phase of attachment and eruption. Teeth succeeding each other at a particular position, i.e. a functional tooth and all of its predecessors and successors constitute a tooth family (Reif, 1984). In the mediodorsal row and dorsal row the number of teeth belonging to one tooth family never exceeds two, and frequently only one tooth is present. There are, however, major differences in the number of tooth family members in the ventral row, depending on the age of the fish (Van der Heyden et al., 2000). In young zebrafish (2–24 days post-fertilization [dpf]),

**FIGURE 1** (a) Scanning electron microscope image of dentition on the right pharyngeal jaw of a juvenile zebrafish (Danio rerio) consisting of three rostro-caudal teeth rows. All 11 teeth positions labeled, from rostral to caudal, according to their ventral row positions (1V–5V): mediodorsal row (1MD–4MD), dorsal row (1D and 2D). Tooth in position 4 MD is shed. Scale bar = 500 μm (Van der Heyden et al., 2000; reproduced with permission of the editor). (b) Schematic drawing of adult zebrafish pharyngeal jaws. Dorsal slightly posterior view of jaws displaying functional teeth (Huysseune et al., 2005). (c) Right pharyngeal jaw from Figure 1(b) tilted over 90° (Huysseune et al., 2005; reproduced with permission of the editor).
the attachment of a replacement tooth is often dissociated from resorption of its predecessor, and therefore a tooth family frequently consists of three members, and infrequently of four members (two functional teeth, a tooth in resorption, and a developing tooth). In older juveniles and adults (from approximately 24 dpf onwards) frequently one or two, maximum three members of a tooth family are present (a tooth in resorption, a functional tooth and a replacement tooth) (Van der Heyden et al., 2000).

Here we artificially retarded the development of zebrafish through inducing deficiency of thyroid hormones by thiourea treatment. Thiourea, a goitrogen, inhibits the synthesis of thyroid hormones (triiodothyronine - T₃ and thyroxine - T₄) and thereby causes a deficiency of thyroid hormones (Bern & Nandi, 1964). Both T₃ and T₄ are main regulators of fish development (Blanton & Specker, 2007; Janz & Weber, 2000; Shkil, Kapitanova, Borisov, Abdissa, & Smirnov, 2012). Here the question is addressed whether changing the developmental rate induces dental variability in a cyprinid species known to have a static tooth formula. In particular, given the low intraspecific variability in the dentition of wild-type zebrafish, suggesting severe developmental constraints that prevent changes in tooth patterning, the further question rises whether supernumerary teeth can occur. We hypothesize that retarded development will result in a modification of the tooth formula and tooth replacement pattern in a significant number of specimens. This hypothesis is based on the results from previous studies describing the effects of alterations of the developmental rate, notably on the dentition of L. intermedius (Shkil et al., 2010), and the caudal fin and the Weberian apparatus of L. intermedius and D. rerio (Kapitanova & Shkil, 2014a,b).

2 | MATERIALS AND METHODS

2.1 | Animals

Wild-type zebrafish were maintained under laboratory conditions in accordance with Westerfield (2000). Fertilized eggs were obtained by natural spawning; the clutch was divided into equal numbers for control and treated groups. Eggs were placed in 40-L glass aquaria with dechlorinated and UV-treated tap water (pH 7.1–7.4) with permanent aeration, at a temperature of 28.0 ± 0.5°C, and a light regime of 12 hr daylight/12 hr night. Eggs of the treated group were exposed from fertilization onwards to 0.02% thiourea (NH₂CSNH₂, State Standard 6244-73), with 1/3 of the solution exchanged daily. Larvae were fed nauplii of Artemia salina and artificial food (Tetramin baby, Tetra, Germany), juveniles received A. salina and Tetramin Junior (Tetra, Germany). A total of 144 larvae (70 control and 74 thiourea-treated fish, ages between 5 and 18 dpf) and 66 thiourea-treated juveniles (ages between 41 and 59 days dpf) were euthanized with an overdose of the anesthetic lidocaine (2-(diethylamino)-n-(2, 6-dimethylphenyl) acetamide). Standard length was measured from the anterior tip of the snout to the posterior tip of the hypurals up to the nearest 0.1 mm (±0.1 mm) (Önsoy, Tarkan, Filiz, & Bilge, 2011). The fish were fixed and stored in 4% buffered formaldehyde until staining and clearing.

2.2 | Staining and clearing

The 66 juvenile specimens were stained and cleared according to an acid-free whole mount cartilage and bone staining protocol (Walker & Kimmel, 2007), using a staining solution composed of two parts. Part A contained 0.02% alcian blue, 60 mM MgCl₂ and 70% ethanol. Part B contained 0.5% alizarin red S powder dissolved in demineralized water. All treatment steps were carried out at room temperature. Prior to staining, the fixative was removed by rinsing in tap water for 10 min, and the samples subsequently partly dehydrated in 50% ethanol for 10 min. The ethanol was removed and the juvenile zebrafish were stained overnight in a mixture containing 10 μl staining solution part A per 1 ml staining solution part B, mixed just prior to staining. Specimens were cleared by rinsing in demineralized water for 10 min. Pigmentation was removed with a bleaching solution containing 1.5% H₂O₂ and 1% KOH in tubes with the lids open, for 20 min. After removing the bleaching solution the tissue was cleared overnight by a clearing solution containing 20% glycerol and 0.25% KOH. This was replaced by a solution containing 50% glycerol and 1% KOH until dissection. Dissected jaws were stored in 100% glycerol.

2.3 | Description of tooth pattern

Both jaws were dissected out from each individual. The number, position and stage of the teeth were examined using a stereomicroscope (Zeiss, Axio Zoom V16) and a compound microscope (Zeiss, Axio Imager Z1) equipped for brightfield and epifluorescence (wavelength 551–573 nm). The position of the teeth and their developmental stage were recorded and visualized in dentition schemes (Figures 2 and 3). Teeth were identified based on their position relative to the 11 regular tooth positions in each of the three rows, from rostral to caudal, as either belonging to the ventral row (1V–5V), mediiodorsal row (1MD–4MD), or dorsal row (1D and 2D), as described by Huysseune, Delgadoa, and Witten (2005) (Figure 1).

Teeth were furthermore assigned to one of four stages in their life cycle: (i) functional teeth, (ii) teeth in resorption, (iii) replacement teeth with only the mineralized tip visible, and (iv) replacement teeth in the process of attachment. The latter could be identified by the presence of a translucent collagenous matrix below the tooth base, extending towards the supporting bone. Functional teeth were ankylosed to the supporting bone by a mineralized attachment bone. Teeth in resorption displayed demineralized areas at the base, usually combined with a serrated edge of the tooth base and a hypermineralized (and therefore translucent) enameloid cap. Finally, a tooth was recorded as missing when an enlarged distance between two regularly positioned teeth was observed, either combined with the presence of a cavity in the supporting bone, or with the presence of a successor.
Supernumerary teeth were assigned to two categories: (i) supernumerary teeth anterior to a tooth row (STAs) and (ii) supernumerary teeth lateral to a tooth row (STLs). In this study a tooth was considered to be "regular" (i.e. not supernumerary) when it was present as: (i) a functional tooth, (ii) a tooth in resorption, or (iii) a functional tooth ventral to a tooth in resorption, in 1 of the 11 regular tooth positions. Likewise, a tooth was considered to be "regular" when it was present as a replacement tooth positioned ventrally to its predecessor. A tooth located in other positions (e.g. in front of the first regular tooth position of a tooth row) or combined with another functional tooth of the same tooth family, was considered to be a supernumerary tooth. Supernumerary teeth were defined in a very conservative way. Thus, when a tooth in a regular position showed only very slight signs of resorption, a functional tooth positioned ventral to the latter was not considered as supernumerary because we wanted to exclude an influence of minor variation in timing of tooth resorption on the results. It should be noted that mineralized structures are colored by alizarin red S used in the staining procedure and therefore teeth in a phase of initiation, morphogenesis and early cytodontification, i.e. before deposition of mineralized matrix, were not visualized.

2.4 | Dental formulae

The dental formula describes the number of teeth in a tooth row from the outermost row on the left side to the outermost row on the right side; a hyphen denotes the transition from the left to the right side. The dental formula from wild-type zebrafish is 2.4.5–5.4.2 (Stock, 2007) (Figure 1). For the description of the dental formulae in the present study, regularly positioned teeth and supernumerary teeth anterior to a regularly positioned tooth (STA) were taken into account.

2.5 | Data analysis

Data were analyzed using the statistical software WinSTAT (R. Fitch Software). Normal distribution was tested by the Kolmogorov-Smirnov test for continuous variables and the chi-square test for categorical variables. Pairwise comparisons were carried out using
the t-test for independent samples when the data appeared to be normally distributed; for other pairwise comparisons the parameter-free U-Test was used. An ANOVA was carried out for analyzing the correlation between standard length and age of the fish.

3 | RESULTS

3.1 | General aspects

A pairwise comparison (U-test) of the SL of 70 thiourea-treated zebrafish and 74 non-treated fish of the control group (age group 5 dpf–18 dpf) revealed no statistically significant differences in SL (p > .05). Thiourea-treated zebrafish younger than 18 dpf, tended to be even larger than non-treated specimens. The standard length (SL) from the 66 thiourea-treated experimental specimens ranged between a minimum of 7.5 mm and a maximum of 12.4 mm. A trend towards an increase in standard length with increasing age was observed with an average of 8.7 mm at 41 dpf to 11.3 mm at 59 dpf. An ANOVA nevertheless revealed no significant differences in standard length between the age groups (p > .05). Nonetheless, several skeletal structures displayed a developmental retardation, such as a delay in the development of supraneurals and ribs, a delay in ossification of neural spines, hemal spines, and parapophyses in 61 of the 66 treated specimens. In particular, retardation of the caudal fin endoskeleton was prominent, as revealed by comparison to the developmental table provided by Bensimon-Brito, Cancela, Huysseune, and Witten (2012) (data not shown). These data are consistent with earlier published reports regarding the effects of thiourea treatment in cyprinids, and its qualification as heterochrony (Bolotovskiy & Levin, 2011; Levin, Bolotovskiy, & Levina, 2012; Shkil et al., 2010).

Both pharyngeal jaws of all 66 thiourea-treated fish were examined. The total number of teeth ranged from 24 to 38. Functional teeth and replacement teeth were present in all 66 specimens; in 44 fish teeth also in resorption were observed. The minimum, maximum, and average number of observed teeth are reported in Table 1.

The thiourea treatment resulted in a high degree of variability in the dentition. In about half of the specimens, the tooth pattern did not correspond to the standard pattern as described for untreated wild-type zebrafish (Huysseune et al., 2005). In particular, supernumerary teeth anterior to the first regular tooth of a tooth row (i.e. anterior to 1V, 1MD or 1D) (STAs), functional teeth lateral to a regular tooth position (STLs), and left–right asymmetries were observed.
3.2 | Supernumerary teeth

In total, 37 specimens displayed supernumerary teeth. Two categories of supernumerary teeth were observed. Supernumerary teeth anterior to the first regular tooth position of a tooth row (STAs) (Figures 2 and 4) were found in 25 specimens, corresponding to the number of fish with aberrant tooth formulae (s3). Supernumerary teeth were also located lateral to regularly positioned teeth (STLs) (Figures 3 and 4b), the case in 22 specimens. In approximately one-third of all specimens with supernumerary teeth (13 of 37) both categories, STA as well as STL, were present. In general, both categories were observed in all three rows of teeth, but the frequency of appearance was tooth row dependent. A majority of STAs was found in the mediodorsal row, followed by the ventral row. STAs appeared very infrequently in the dorsal row. In contrast, most STLs were recorded for the ventral tooth row, followed by the mediodorsal row, and rarely the dorsal row (Table 2).

There was a trend of an increase in frequency in the presence and number of supernumerary teeth with increasing age. Compared to young individuals, older individuals showed supernumerary teeth more frequently, and the number of supernumerary teeth was higher. Older individuals regularly displayed both, i.e. an extra tooth in a row as well as an extra tooth row.

The supernumerary teeth appeared in a certain sequence during ontogeny. The first supernumerary teeth to emerge were located anterior to the mediodorsal row and ventral row, followed by supernumerary teeth lateral to regularly positioned teeth. The last supernumerary teeth to appear were observed anterior to the dorsal tooth row.

In about two-thirds of the specimens with additional teeth (26 of 37), supernumerary teeth were present on one jaw only, with no obvious dominance for the left or the right. A pairwise U-test revealed no significant differences between the presence and number of supernumerary teeth in the left and right jaw ($p > .05$), thus excluding directional asymmetry.

3.3 | Characteristics of supernumerary teeth

Supernumerary teeth anterior to the first regular tooth of a tooth row (STAs) were in various stages of development (19 functional teeth, 3 replacement teeth in late cytodifferentiation stage close to the time-point of attachment, seven mineralized replacement teeth in a relatively early developmental stage with the tip of the tooth visible only,
and seven teeth in resorption). There was no apparent relationship between the stage of development or functionality of the STA and of the regular tooth posterior to it. Attached STAs occurred only from 47 dpf onwards. STAs were placed equidistantly with the regular teeth in a row. When present as a functional tooth the shape of a STA appeared to be slightly concave and hook-shaped, but more conical compared to the anteriormost tooth in their respective tooth row (Figure 4a).

Supernumerary teeth lateral to a tooth row (STLs) and the corresponding regular tooth on that particular tooth position were closely spaced (Figure 4b). Shape and size of the closely-spaced teeth appeared to be similar and the supporting bone appeared to be broadened in the attachment area. Occasionally a successor was observed ventrally to a STL (two individuals).

### 3.4 | Dental formulae

Among the 66 individuals, 41 fish had the typical dental formula for D. rerio (2.4.5–5.4.2) (note that the dental formula excludes STLs and therefore this number differs from the number of fish with a standard pattern). The remaining 25 fish displayed nine aberrant formulae, usually differing between the left and right side. The most common aberrant formulae were 2.4.5–5.5.2 (six specimens) and 2.5.5–5.4.2 (eight specimens) (Table 3).

### 4 | DISCUSSION

For the current experiment we used zebrafish, a cyprinid with an invariant tooth formula (Huysseune, Soenens, & Elderweirdt, 2014; Van der Heyden et al., 2000), and artificially induced a retardation of development by deficiency of thyroid hormones. This study revealed the presence of two distinctive types of supernumerary teeth: supernumerary teeth anterior to the first regular tooth of a tooth row (STAs), i.e. in an ectopic position, prolonging the existing tooth row (Figure 2); and supernumerary teeth lateral to the regular tooth positions (STLs). STLs were suggestive of the formation of an accessory tooth row (Figure 3). For simplicity, the latter will be referred to as accessory tooth row.

The results from this study suggest that the mechanisms underlying the development of the two different types of supernumerary teeth (STAs and STLs) are distinctive. The relatively large distance between supernumerary teeth from type STA and the anteriormost regular tooth of the corresponding row (Figure 2) suggests an independent initiation mechanism, not linked to irregular maintenance of the tooth family, such as traumatic tooth splitting or fractured teeth, or placode splitting. In contrast, STLs were shaped similarly to the adjacent regular tooth (Figure 4b). This similarity, along with the close spacing of these teeth, suggests that both the regular tooth and the adjacent STL, are members of a single tooth family. The likely presence of two members of a tooth family in the same functional state can be a consequence of the significant retardation of tooth life cycle caused by deficiency of thyroid hormones, as co-functionality within a tooth family is a common characteristic in the ventral tooth row of D. rerio younger than 24 dpf.

Interestingly, these results are partially consistent with studies on D. rerio subjected to a high level of retinoic acid (Gibert et al., 2015; Seritrakul et al., 2012) or a high level of Fgf10 (Jackman et al., 2013). Gibert et al. (2015) reported the presence of an additional tooth in the ventral row, posterior to the tooth position 5V, in wild-type zebrafish (cyp 26b1+/+) treated with all-trans retinoic acid,

### Table 3: Variant tooth formulae observed in pharyngeal dentition of thiourea-treated zebrafish (n = 66)

<table>
<thead>
<tr>
<th>Dental formula</th>
<th>No. specimens</th>
<th>Symmetric</th>
<th>Asymmetric</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4.5–5.4.2</td>
<td>41</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>STAs not present</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.4.5–5.5.2</td>
<td>6</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>2.4.5–5.5.3</td>
<td>1</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>2.4.5–6.4.2</td>
<td>2</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>STAs located on right jaw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.4.6–5.4.2</td>
<td>2</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>2.5.5–5.4.2</td>
<td>8</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>STAs located on left jaw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.4.6–6.4.2</td>
<td>1</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>2.5.5–5.5.2</td>
<td>3</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>2.5.5–6.6.2</td>
<td>1</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>3.6.5–6.4.2</td>
<td>1</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>STAs located on both jaws</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Specimen ages ranged from 41 days postfertilization (dpf) to 59 dpf. Standard lengths ranged from 7.5 to 12.4 mm (note that the dental formula excludes STLs, therefore the number of specimens with dental formula 2.4.5–5.4.2 differs from number of fish with standard pattern). X indicates if the two jaw halves have a symmetric or asymmetric dental formula.
and for the heterozygous adult zebrafish mutant stocksteif (sst) (cyp 26b1+/−). The gene cyp 26b1 encodes for an enzyme involved in the degradation of retinoic acid (Fransén et al., 2013) and thus the zebrafish mutant stocksteif possesses dentitions similar to wild-type zebrafish treated with exogenous retinoic acid. The digitalized microtomography image of the dentition of an adult stocksteif mutant, shown in the article, indeed reveals a supernumerary tooth in the ventral row, albeit that the tooth is located anterior to 1V, not posterior to 5V. It is consistent in shape and position with supernumerary teeth from the type STA observed in the present study.

Jackman et al. (2013) revealed the presence of supernumerary teeth in zebrafish treated with Fgf10 and in the transgenic zebrafish Tg(fishp70l:fgf10a-GFP)12. These authors reported the presence of closely spaced supernumerary teeth in a single specimen that correspond in location, shape and attachment pattern to supernumerary teeth from type STL in this experiment. Jackman et al. (2013) furthermore reported an asymmetric tooth pattern and found supernumerary teeth in ectopic locations. In contrast to the results of this study, these ectopic teeth were not restricted to the fifth ceratobranchial.

Partial similarities in the results between the studies of Jackman et al. (2013), Gibert et al. (2015), and this study, may not be coincidental, and perhaps point to a potential mode of action. It is known that, e.g. during cranial neural crest development (the source of dentin producing odontoblasts), thyroid hormones (TH) and retinoic acid (RA) present a tight spatiotemporal regulation. This regulation is mainly mediated by retinoid X receptor signaling (Bohnsack & Kahana, 2013). Retinoid X receptors are shared heterodimeric partner for thyroid hormone receptors and retinoic acid receptors. TH and RA have opposite effects on pharyngeal arch development and co-regulate gene expression by repressing the activation of each other’s target (Bohnsack, Gallina, & Kahana, 2011; Bohnsack & Kahana, 2013). One can suggest that deficiency of thyroid hormones induces a shift in gene expression by favoring response elements for retinoic acid. This in turn would alter processes in posterior pharyngeal arch development and retard the development of the dentition. As a consequence, phenotypes obtained after excess of retinoic acid could resemble phenotypes of individuals subjected to deficiency of thyroid hormones. While speculative, this scenario at least offers a hypothesis that can be tested in future studies. An alternative mechanism for the presence of supernumerary teeth is that TH deficiency may lead to postponed resorption of the functional teeth, given that under physiological conditions, hypothyroidism is known to cause reduction in osteoblast formation and osteoclast resorption (Siddiqui & Partridge, 2016).

The changes in the developmental rate in the current experiments resulted in an unexpected high number of variant tooth formulae (Table 3). This raises the question of whether the presence of supernumerary teeth, which were observed during this experiment, mirrors a heterochronic shift towards an ancient tooth pattern. There has long been a debate on the plesiomorphic number of pharyngeal tooth rows in cyprinid fishes (Ahnelt, Bauer, Löfler, & Mwebaza-Ndawula, 2006; Pasco-Viel et al., 2010; Stock, 2007; Zhu, 1935). Most authors consider a three-row dentition as the ancestral dental state in cyprinids (Pasco-Viel et al., 2010; Zhu, 1935). The tooth formula of zebrafish (2.4.5–5.4.2) is regarded as the ancestral cyprinid tooth formula (Stock, 2007; Zhu, 1935).

Under natural conditions some cyprinid species exhibit either three or four rows of teeth (Eastman & Underhill, 1973; Shkil & Levin, 2008; Shkil et al., 2010) and the intraspecific variation in number of teeth has been described. This variation has been hypothesized to be occasional abnormalities, resulting from disorders in tooth development and replacement. Alternatively, they have been suggested to be morphological novelties (Eastman & Underhill, 1973; Pasco-Viel et al., 2010).

Golubtsov et al. (2005) hypothesized that the genome of cyprinids contains quiescent genetic information, conserved from ancestors of cyprinids, enabling the development of aberrant number of tooth rows in single specimens (spontaneous atavism) or as a regularly occurring phenomenon in some cyprinid species (taxic atavism). The presence of more than three rows of teeth in ancestral cyprinids is also supported by Ahnelt et al. (2006), who reported four, occasionally five, rows, for all 42 specimens of Rastineobola argentea captured in the northern part of Lake Victoria. The appearance of supernumerary teeth in our experimental fish allows us to conclude that the zebrafish genome has the capability to produce supernumerary teeth and likely also extra rows of teeth. The same potencies were described for the large African barb (L. intermedius) (Shkil et al., 2010) and roach (Rutilus rutilus) (Bolotovskiy & Levin, 2011). In both species the incidence for supernumerary teeth was about 20% when development was pharmacologically retarded by thiourea. In contrast to zebrafish, however, both species display a plastic tooth formula under natural conditions. In a natural population of large African barb (L. intermedius) in the Ethiopian Lake Tana, a fourth row of teeth was reported in about 10% of the examined specimens (Shkil et al., 2010).

The appearance of supernumerary teeth and extra tooth rows in natural populations of different groups of cyprinids (Danioninae, Barbinae, Cyprininae) as well as in experiments, indicate that the supernumerary teeth are to be considered an atavistic feature rather than a morphological novelty or the result of an occasional developmental abnormality. The experimental data demonstrate that heterochronies can be a potent source of cyprinid morphological diversity.

REFERENCES


