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# The moment of tooth: rate, fate and pattern of Pacific lingcod dentition revealed by pulse-chase

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Tooth replacement rates of polyphyodont cartilaginous and bony fishes are hard to determine because of a lack of obvious patterning and maintaining specimens long enough to observe replacement. Pulse-chase is a fluorescent technique that differentially colours developing mineralized tissue. We present *in situ* tooth replacement rate and position data for the oral and pharyngeal detentions of *Ophiodon elongatus* (Pacific lingcod). We assessed over 10 000 teeth, in 20 fish, and found a daily replacement rate of about two teeth (3.6% of the dentition). The average tooth is in the dental battery for 27 days. The replacement was higher in the lower pharyngeal jaw (LPJ). We found no difference between replacement rates of feeding and non-feeding fish, suggesting feeding was not a driver of tooth replacement. Lingcod teeth have both a size and location fate; smaller teeth at one spot will not grow into larger teeth, even if a large tooth nearby is lost. We also found increased rates of replacement at the posterior of the LPJ relative to the anterior. We propose that lingcod teeth do not migrate in the jaw as they develop; their teeth are fated in size and location, erupting in their functional position.

## 1. Background

Vertebrate dentitions fall into two loose categories—diphyodont dentitions with a single set of replacement teeth, and polyphyodont dentitions where teeth are replaced continuously. Genetic pathways for tooth development are conserved across both types of dentition [1,2], and the diversity of tooth shapes and dental batteries lie in differential regulation of the same genetic toolbox [3,4]. Some polyphyodonts have fated replacement teeth, where an individual tooth is destined to replace a functional tooth at a specific location [2]. Sharks present an interesting example of tooth fate because though the teeth are morphologically similar, they nevertheless have a distinct identity. Each functional tooth has a file of replacements lined up behind it [5,6]. Bony fishes offer many examples where it is difficult to see a replacement tooth having an identity relative to a functional tooth, the non-fated condition. For example, in the fine, dense, spatulate dentition of loricariid catfishes, any particular well-hooked tooth is much like any other, and replacements come in from behind without a clear link to an old tooth [7]. It remains to be determined whether morphologically homodont teleosts have identifiable replacement teeth that will emerge to fill a particular position or role in the dental battery [8].

A fundamental question in polyphyodont dentitions is whether replacement is driven by damage [9,10]—does a new tooth grow in response to a broken tooth, or does it appear because certain regions of the jaw experience greater mechanical stress [8,9,11]? This complexity is best showcased in the *en bloc* replacement of one-quarter of the entire dental battery by piranhas [11]. This maintains the integrity of an interlocked, sharp and serrated dental battery, and certainly is not a response to every tooth becoming damaged. However, we do not know whether it is a

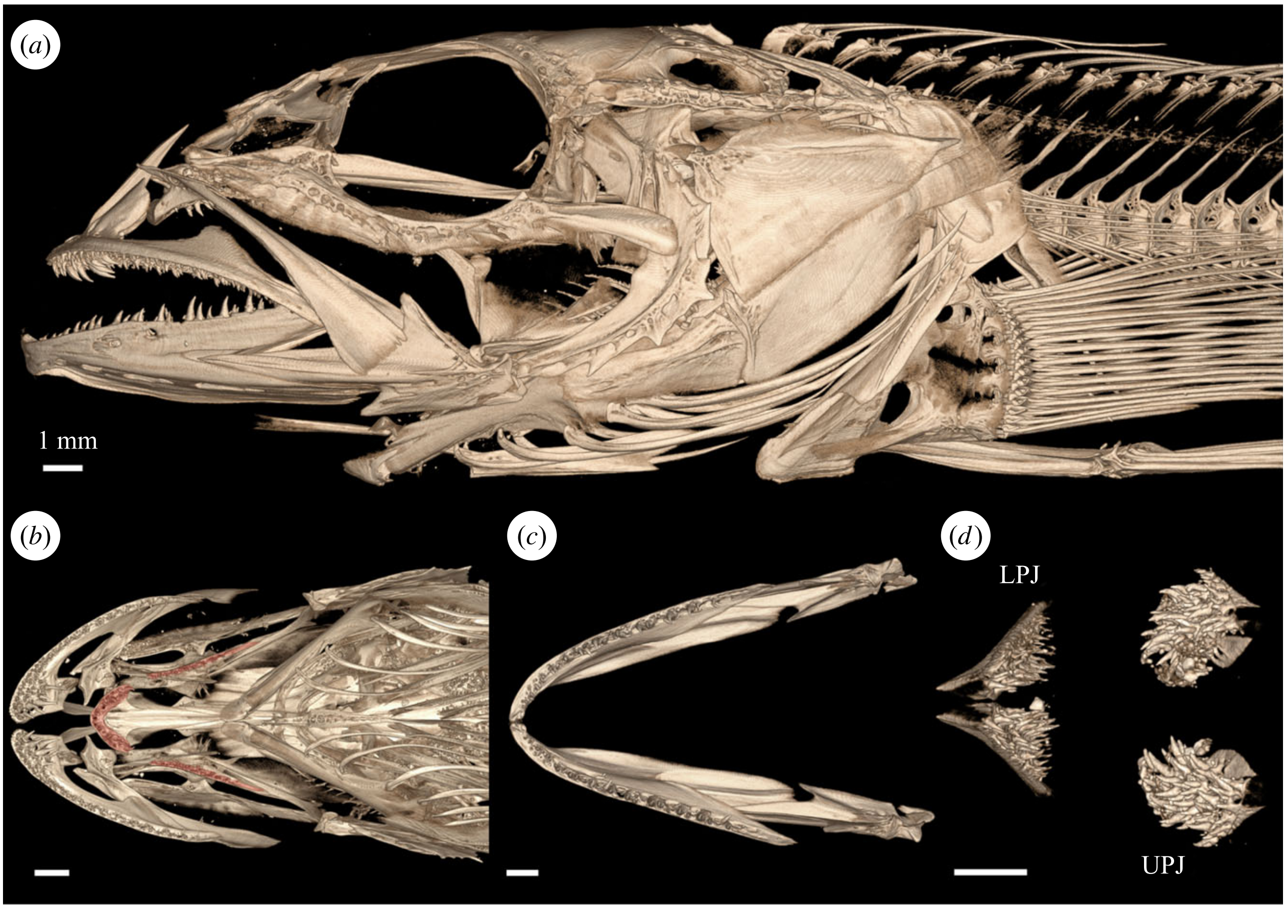

**Figure 1.** Volume rendering of *O. elongatus* showing toothed bones: (*a*) lateral view of the skull; (*b*) ventral view of the premaxilla, maxilla, vomer (red) and palatine (red) with the lower jaw and urohyal removed; (*c*) dorsal view of the dentary and lower jaw; and (*d*) lower (LPJ) and upper (UPJ) pharyngeal jaws. Scale 1 mm. (Online version in colour.)

response to a single tooth being broken. An advantage of continuously replacing teeth is that they can occupy a risky region of the *wear resistance* ←→ *performance* continuum [9,12].

Bony fishes usually have two sets of dentitions, one on their oral jaws and a second, often functionally divergent set, on their pharyngeal jaws. Each has its own presumed functional role; oral jaws grab prey, and pharyngeal jaws process and crush food [13–17]. We investigate tooth replacement in the Pacific lingcod (*Ophiodon elongatus*)—an easily fished and housed marine piscivore that requires a sharp and full dentition to successfully capture, manipulate and process prey. As juveniles, lingcod experience a change in diet over ontogeny based on prey scale that is paired with a shift in reliance on vomerine teeth in juveniles to greater usage of premaxillary teeth in adults [13].

The goals of the study were five-fold: (i) quantify the replacement rate of teeth in oral and pharyngeal jaws; (ii) highlight tooth replacement hotspots; (iii) compare replacement between oral and pharyngeal jaws; (iv) determine whether a tooth is fated to replace a similarly sized tooth; and (v) compare replacement in feeding versus fasting fish. Polyphyodont dentitions are thought to be lost due to the high metabolic cost of generating an expensive material—enamel [4]. If this is true, we should see lower rates of replacement in starved versus fed lingcod. Also, juvenile lingcod rely on different tooth-bearing bones for prey capture and prey processing [13]. Therefore, we expect to see high rates of replacement, and larger replacement hotspots on bones used more by juvenile fish. Finally, lingcod dentitions are morphologically heterodont but functionally homodont, meaning teeth of differing size function similarly [15]. We predict that large and small conical teeth are replaced at similar rates and modes on the oral jaws.

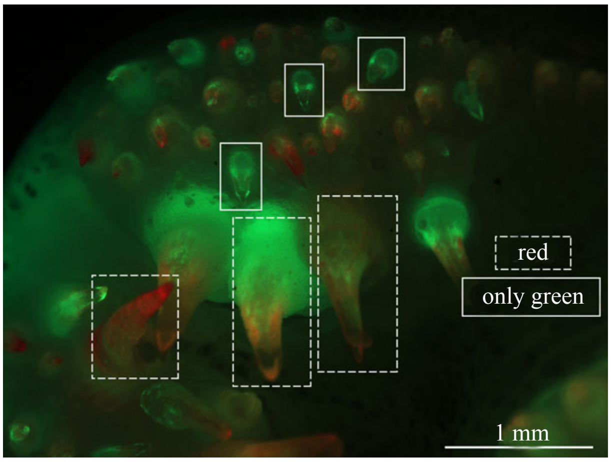

**Figure 2.** Variations of fluorescence. Old teeth, those that pre-dated the calcein dye, are at least partially red (dashed outline). New teeth, those that were replaced after the alizarin dye, are only green (solid outline). (Online version in colour.)

## 2. Methods

We collected Pacific Lingcod, *O. elongatus* ($n = 30$), by seining at Jackson Beach, San Juan Island, WA, USA. We housed fish in 108 l, flow-through sea tables with 10–15 cm rocks to allow fish a place to hide.

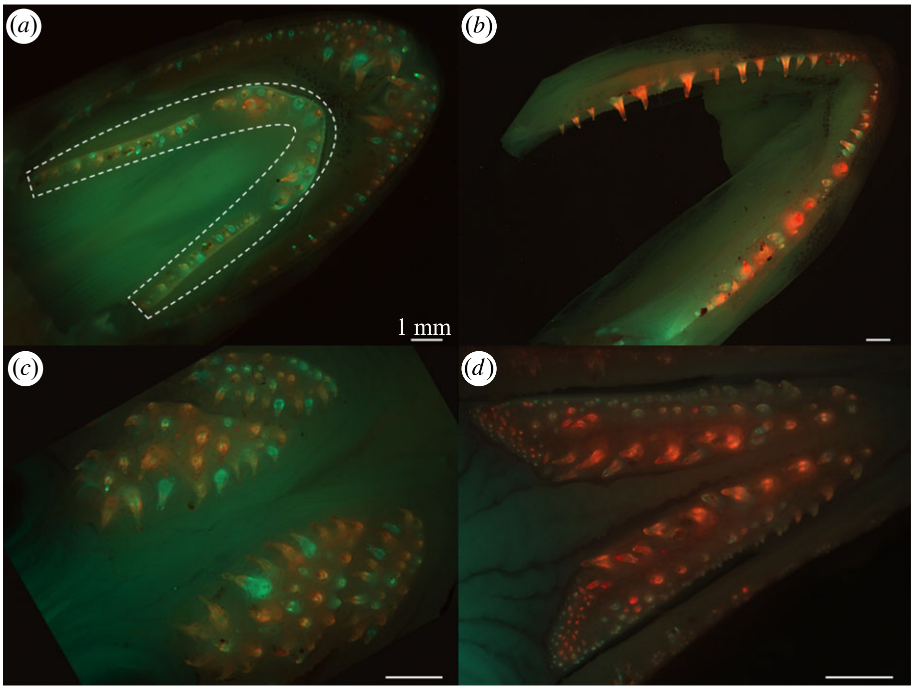

**Figure 3.** Fluorescent microscopy images of each toothed element showing old teeth (at least partial red fluorescence) and newly replaced teeth (green fluorescence): (*a*) vomer (includes palatine, surrounded by dashed line), (*b*) dentary, (*c*) upper pharyngeal jaw and (*d*) lower pharyngeal jaw. All toothed elements with the anterior towards the top right corner. Scale 1 mm. (Online version in colour.)

**Table 1.** Replacement data for both rate ($n = 10$) and feeding experiments ($n = 10$).

| experiment | number of specimens | total length (mm) | number of teeth censured | average replacement rate (per day) |
|---|---|---|---|---|
| rate experiment | 10 | 134–151 | 5436 | 3.6% ± 0.14 |
| feeding group | 5 | 127–142 | 2551 | 3.6% ± 0.23 |
| non-feeding group | 5 | 117–150 | 2593 | 3.9% ± 0.20 |
| feeding experiment | 10 | 117–150 | 5144 | 3.7% ± 0.15 |
| all fish combined | 20 | 117–151 | 10 580 | 3.7% ± 0.10 |

For pulse-chase experiments, we removed fish ($n = 10$, TL 134–151) from normal housing to a covered 20 l tank filled with a 0.05% alizarin red S solution (3,4-dihydroxy-9,10-dioxo-2-anthracene sulfonic acid sodium salt, CAS 130-22-3). Unlike Ellis *et al.* [18], no buffer was required to maintain a neutral pH because seawater is naturally buffered. We placed the 20 l tank into a tub of continuously flowing seawater to maintain a constant temperature and covered it with a black tarp to block out light. We put lingcod ($n = 10$) in the alizarin solution for 12 h before returning them to normal conditions. At 2-day intervals, two specimens were chosen at random from the tank and put in a light-tight 80 l tank with a 0.005% calcein (bis[*N*,*N*-bis(carboxymethyl)aminomethyl] fluorescein, CAS 154071-48-4) solution. We maintained the lingcod in the calcein dye for 12 h, then returned them to a flow-through holding tank to flush excess calcein before euthanasia in $250\ \mathrm{mg\ l^{-1}}$ MS-222 (ethyl 3-aminobenzoate methanesulfonate, CAS 886-86-2). Specimens were fixed in 10% buffered formalin for 12 hours. The premaxilla, vomer (including the palatine), dentary and upper pharyngeal jaw and lower pharyngeal jaw (LPJ) were dissected and stored in a dark container of 95% ethanol until imaged.

## (a) Tooth wear experiments

We put an additional 10 specimens (TL 117–150 mm) through the same alizarin red S and calcein *in situ* experiments but instead of removing fish every 2 days, we divided individuals into two groups: feeding and non-feeding. We put five fish in normal seawater conditions and fed them dead Pacific sand lance (*Ammodytes personatus*). *Ophiodon elongatus* will not eat dead, immobile prey, so dead *A. personatus* were loosely tied with fishing line and placed near the inflowing water current to simulate swimming. Each lingcod was fed one prey fish every 4 days. We put the remaining five fish in a separate tank and did not feed them. After 10 days, both groups were separately placed in a dark tank with a 0.005% calcein solution.

## (b) Imaging analysis

We counted and visualized old teeth and newly replaced teeth on the premaxilla, vomer palatine, dentary, and upper pharyngeal jaw and LPJ (figure 1) using a ZEISS SteREO v20 Discovery microscope (Zeiss Oberkochen, Germany) and X-Cite series 120 fluorescent lamp. Newly replaced teeth, those that had yet to erupt when specimens were stained in alizarin red, emitted only green fluorescence (figure 2). Old teeth, those present during alizarin staining, had at least partial red fluorescence. Photographs of teeth were taken using AxioVision software and a Zeiss AxioCam HRc microscope camera.

**Table 2.** Average percentage replacement per day (2–10 days between treatments), feeding and non-feeding specimens (10 days between treatments).

| | premaxilla | vomer | dentary | upper pharyngeal jaw | lower pharyngeal jaw | average |
|---|---|---|---|---|---|---|
| rate experiment ($n$ =10) | 3.0% ± 0.19 | 3.4% ± 0.23 | 4.0% ± 0.31 | 2.6% ± 0.18 | 4.8% ± 0.37 | 3.6% ± 0.14 |
| feeding ($n$ = 5) | 3.4% ± 0.31 | 3.2% ± 0.30 | 2.5% ± 0.37 | 3.6% ± 0.74 | 5.3% ± 0.31 | 3.6% ± 0.23 |
| non-feeding ($n$ = 5) | 4.9% ± 0.67 | 3.4% ± 0.31 | 3.5% ± 0.15 | 3.5% ± 0.15 | 4.1% ± 0.36 | 3.9% ± 0.20 |

### (c) Data analysis

We calculated the rate of replacement by dividing the percentage of new teeth (green) by the number of days between alizarin and calcein treatments, assuming constant tooth replacement. Additionally, we measured replacement frequency for feeding and non-feeding individuals ($n$ = 10) by calculating and mapping the percentage of specimens that had replaced a tooth in a certain position. Regions of great replacement frequencies, 60% or higher, are ‘hotspots’.

We used an ANOVA to check for significant differences in replacement rates between right and left dentitions before binning data for further analyses. To measure the rate of replacement, we performed two separate analyses. The first answers the question: do larger fish replace more teeth than smaller fish? We used a linear regression analysis to compare the rate of replacement to the total lengths of each individual. Next, we asked if tooth replacement was faster on different bones. A Shapiro–Wilk test on the binned replacement rate data showed a normal distribution of tooth replacement rates except for the premaxilla in non-feeding fish. We used an ANOVA to test if the rate of tooth replacement varied among toothed elements [19] and subsequent Tukey HSD to summarize replacement rate of the *in situ* pulse-chase. To test if replacement rate was affected by wear, we used an ANOVA and Tukey HSD between feeding and non-feeding groups. All statistical analyses were completed in R (v. 4.0.2) with a significant *p*-value threshold of 0.05.

## 3. Results

On the five tooth-bearing bones in the mouth of the Pacific lingcod, there are 555 ± 24.4 teeth and 3.6% are replaced each day (table 1 and figure 3). The dentary, with 62 ± 6.5 teeth, replaces 4.0% of its dentition, or two teeth per day, which is 32% more than the premaxilla (3.0% ± 0.9). Together the pharyngeal jaws have 60% of the total dentition, an average of 335 ± 28.3 teeth. The premaxilla and dentary lose 8 ± 2.0 fewer teeth than the pharyngeal jaws. The tooth-bearing bones braced by, or part of, the neurocranium (premaxilla, vomer, palatine and upper pharyngeal jaw) lose fewer teeth than the bones that are unsupported (dentary and LPJ) by 10 ± 1.8 per day (table 2 and figure 4). Larger lingcod replace more teeth per day than smaller ones (electronic supplementary material, figures S1 and S2). Despite feeding lingcod with large, bony prey items, we saw no significant overall difference in replacement rates between feeding and non-feeding groups (figure 5). Although feeding and non-feeding replacement rates varied by up to 1.5% in the premaxilla, the significance threshold was not reached between groups for any individual toothed bone. The variability in replacement rate was high—one non-feeder replaced 59% of its dentition over the 10 days, but another individual replaced just 29%. There was also no overall significant difference among replacement rates in the rate experiment, feeding and non-feeding groups.

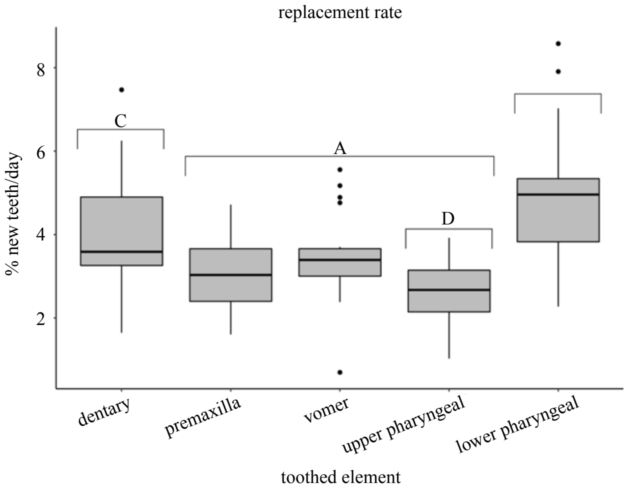

**Figure 4.** Comparison of tooth replacement rates among toothed elements. The replacement rates in the lower pharyngeal jaw (A) were higher than the premaxilla, vomer (includes palatine) and upper pharyngeal jaw (B). Replacement rates of the dentary (C) were also higher than the upper pharyngeal jaw (D).

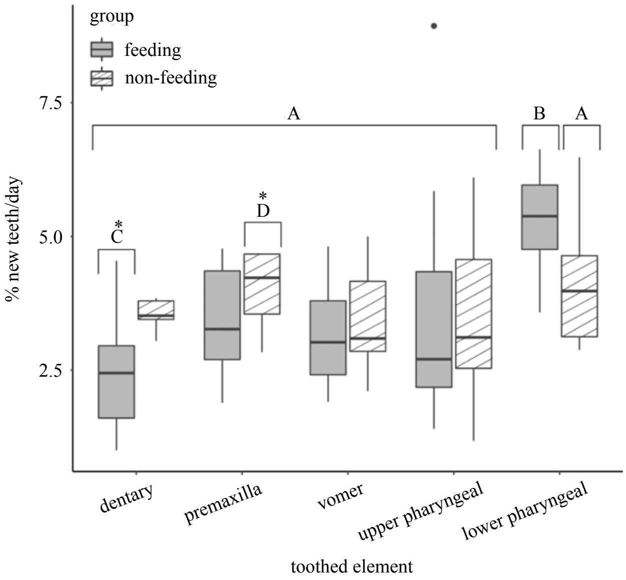

**Figure 5.** Comparison of tooth replacement rates between feeding and non-feeding groups on individual bones. There was no overall difference between the replacement rates between feeding and non-feeding specimens. Teeth in the lower pharyngeal jaw of feeding individuals (B) had higher replacement rates than all other toothed elements (A). Premaxilla of non-feeding individuals (D) had higher replacement rates than the dentary of feeding individuals (C).

Over the course of the 10-day feeding/non-feeding experiment, some tooth locations were consistently replaced among specimens, and these ‘hot spots’ were the same in the

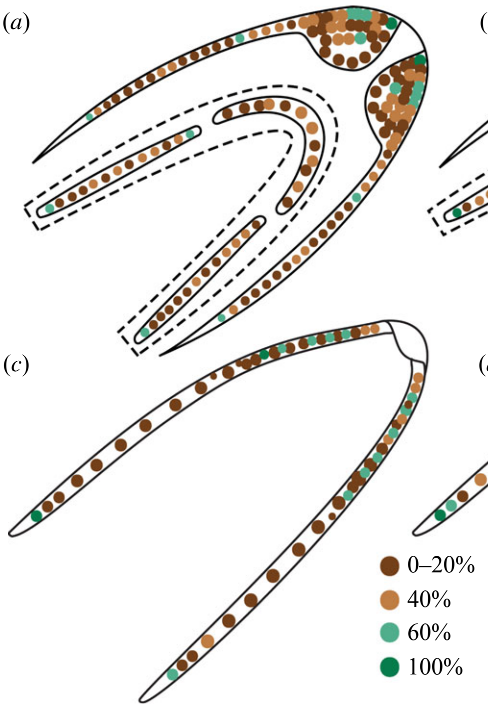

**Figure 6.** Replacement frequency in (*a*,*c*) feeding and (*b*,*d*) non-feeding fish for the premaxilla (*a*,*b*), vomer (includes palatine, dashed outline, *a*,*b*) and dentary (*c*, *d*). (Online version in colour.)

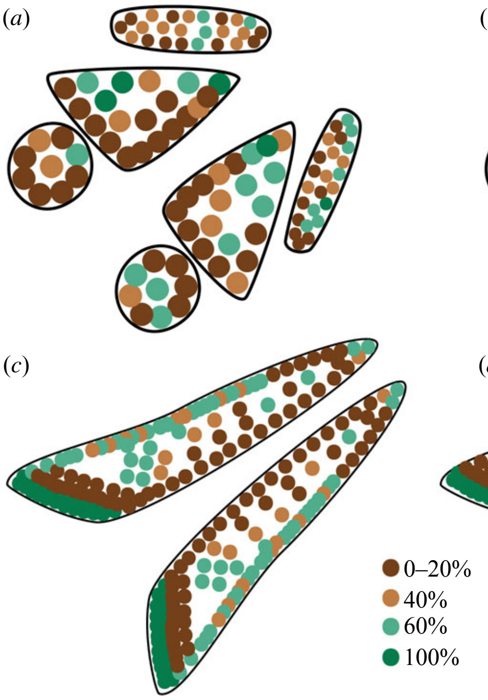

**Figure 7.** Replacement frequency in (*a*,*b*) feeding and (*c*,*d*) non-feeding fish on the upper pharyngeal jaw (*a*,*b*) and lower pharyngeal jaw (*c*,*d*). (Online version in colour.)

two treatments (figures 6 and 7). Anterior premaxillary teeth have the highest replacement frequency; the largest symphyseal canine was replaced 100% of the time while surrounding teeth were replaced 60% of the time or even less often (figure 6*a*,*b*). Posterior premaxillary teeth are replaced less than 40% of the time except for the most posterior tooth on the right and left sides of the jaw. This small tooth is replaced 60% of the time in feeding fishes and 100% of time in non-feeding fishes. The posterior teeth on the vomer and palatine were replaced more frequently than the anterior teeth: 60% compared to 0–40% (figure 6*a*,*b*). Small, anterior dentary teeth are replaced less frequently than small posterior dentary teeth (figure 6*c*,*d*). The upper pharyngeal jaw had many teeth that were replaced in at least 60% of the specimens; however, there was little symmetry between the sides of the jaw (figure 7*a*,*b*). The greatest replacement frequencies are seen in the LPJ where teeth in the two most posterior rows are always replaced while most other teeth are replaced two-thirds of the time (figure 7*c*,*d*). This leads to a condition where the posterior teeth appear new and unworn while the anterior teeth are clearly worn.

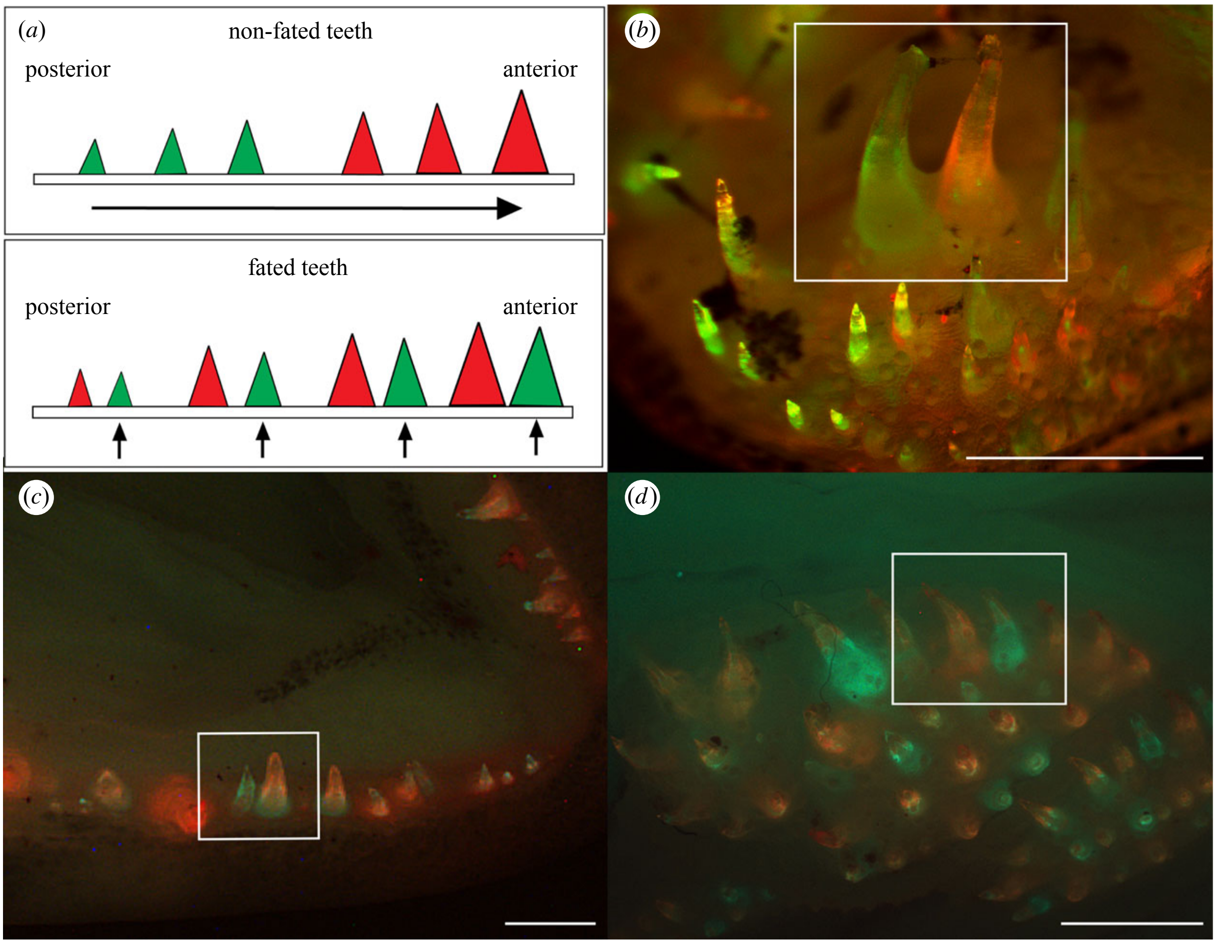

**Figure 8.** (*a*) Fated teeth are in their functional position during eruption and development. (*b–d*) Evidence of fated teeth is seen in all toothed elements with examples shown in the premaxilla (*b*), dentary (*c*) and upper pharyngeal jaw (*d*). Scale 1 mm. (Online version in colour.)

## 4. Discussion

Large teeth erupt as small cones adjacent to the tooth they will eventually replace. Despite being surrounded by many small conical teeth, only one new cone continues to grow to the full size of the caniniform tooth it will replace. Our results stand in stark contrast to the non-fated tooth development pattern in loricariid catfish where replacement teeth show no clear link to a particular old tooth [7] (figure 8*a*). Lingcod teeth are fated; large, new teeth were next to similarly sized old teeth, while small, new teeth were adjacent to the small teeth they were replacing (figure 8). This implies that surrounding small teeth stay small rather than growing to replace the large tooth, and this has implications for the competency of the dental generative tissue to produce different sized cones. Our data suggest that localized regions of the lingcod jaw are competent to produce caniniform teeth, while other areas produce the fine, small cones that aid in gripping and prevent tissue tearing [15]. This system of tooth development makes lingcod a candidate for tissue explant and transplant studies to test the hypothesis that cell–cell pressures mediate the ability of generative tissue to produce teeth of a certain size/shape [20].

The replacement of teeth in cartilaginous fishes, where the functional row is anterior and developing teeth move from generative tissue towards their final position in the dental battery, is a model that has coloured our perception of replacement. This is particularly problematic in the pharyngeal jaws, where small teeth could be seen as immature, growing to replace adjacent larger teeth. Our data refute this notion. The gradation of large to small teeth in the pharyngeal jaw represents the teeth of fated size in positions that do not vary over time (figure 8). The replacement patterns in figure 7 show that small conical teeth at the posterior of the LPJ are replaced more frequently than larger, caniniform teeth at more anterior medial edges, creating a hotspot of replacement. There are good functional reasons for this. The small, slender teeth that catch and grab soft tissue resist bending poorly and should fracture at a high rate [9,12]. A sharp dental battery must be replaced often—in our data small, sharp teeth are in place for just a few days. More robust cones of the piercing teeth are not only less subject to breakage, they are still functional with a dulled tip [21] and in our dataset, they survive several times as long. In dentitions more morphologically heterodont than lingcod, where broad crushing teeth share space with smaller crushing teeth, as well as rounded teeth with small cusps, this replacement pattern makes even more sense [22]. We suggest these dental batteries do not represent an ontogenetic series from young crushers to mature ones, but a gradation of damage susceptibility addressed by variation in replacement rate. The cusped teeth always seem fresher not because they are young teeth about to move to a more stressful position; instead, they are new teeth that have not yet been badly damaged. The spalled surfaces of the large crushing teeth show that damage does not impair function and replacing these useful crushers will not improve the battery [23].

Replacement rates in Pacific lingcod were the same in oral toothed elements and the upper pharyngeal jaw, and significantly higher in the LPJ regardless of whether the fish was feeding or not (figure 4). This implies that neither the cost of generating enamel nor dental wear triggers or controls the replacement rate. Though it has been suggested that teeth have a high metabolic cost, this may not be true in the marine environment where there is no shortage of calcium [4]. We interpret the high replacement rate in the LPJs as evidence of those teeth being at higher risk of breakage over evolutionary time. The lower pharyngeal teeth are ankylosed to highly mobile bones that allow the teeth to impact prey at many different angles. This increases the risk of breakage and so drives a higher replacement rate to maintain a functional dental battery. The homogeneous replacement rates in the rest of the skull are evidence of a similar risk of damage for that entire dentition. Alternatively, it could be that there is a broader tolerance for damage in all but the lower pharyngeal teeth. That is, a few broken teeth on the vomer matter less than on the pharyngobranchials. Regardless, we find no support for hypotheses of damage-driven replacement in this fish, instead, replacement is probably maintained by a spatially and temporally driven developmental network [24–28].

The literature on the development of fish teeth concentrates on species with extreme morphological heterodonty—the fangs of *Trichirus*, lower incisors of clingfishes, the beaks of pufferfish and the interlocking teeth of piranhas and pacus [11,29–31]. In lingcod, the simple conical teeth found on all dentigerous bones vary in size, but this mild morphological heterodonty leads to functional homodonty. Concentrating on fishes with highly specialized, morphologically heterodont dentitions offers a misleading pattern of variation in development across the jaws. In the simple dentition of the lingcod, which we propose is the most common dental morphology in bony fishes, we see a single pattern of tooth replacement. It is more likely that most fishes with many tooth-bearing bones replace their teeth all the same way, while fishes with dissimilar tooth shapes on their oral and pharyngeal jaws have independent replacement patterns and mechanisms.

Ethics. All experiments and animal collection were conducted with permission under a University of Washington animal care and use protocol (IACUC 4238-03).

Data accessibility. All data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.fj6q573vt [32]. The data are provided in electronic supplementary material [33].

Authors' contributions. E.M.C.: data curation, formal analysis, investigation, visualization, writing—original draft; A.P.S.: conceptualization, funding acquisition, project administration, resources, software, supervision, writing—review and editing; K.E.C.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, visualization, writing—original draft, writing—review and editing. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

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