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

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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 \( \rightarrow \) 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.

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.
For pulse-chase experiments, we removed fish \( n = 10, \ TL 134 \text{–} 151 \) 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 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 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 \textit{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 \( (\text{Ammodytes personatus}) \). \textit{Ophiodon elongatus} will not eat dead, immobile prey, so dead \textit{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.
(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.

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
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 6a,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 6a,b).

Small, anterior dentary teeth are replaced less frequently than small posterior dentary teeth (figure 6c,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 7a,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 7c,d). This leads to a condition where the posterior teeth appear new and unworn while the anterior teeth are clearly worn.

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.)
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 8a). 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

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.)
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.

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