

## Review

# Evolutionary Loss of Acid-Secreting Stomach and Endoskeletal Ossification: A Phosphorus Perspective

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**Abstract:** Phosphorus is essential for all life forms on Earth, including eukaryotes (animals, plants, fungi, and protists), prokaryotes (bacteria and archaea), and even viruses. Its significance stems primarily from its presence in nucleic acids (DNA and RNA), where it forms a crucial part of the backbone structure. Beyond this, phosphorus plays a pivotal role in countless biological processes, supporting life at its core. In this article, the author explores the possible causes of stomach loss, focusing specifically on phosphorus absorption, vertebral calcification, and reproduction. Large gonads, characteristic of external fertilization, require substantial amounts of phosphorus for gametogenesis in both females and males, particularly in the latter. This demand has driven the evolutionary calcification of vertebrae, which serve as a phosphorus storage organ. Moreover, to efficiently absorb phosphorus from their diets, shellfish-eating fish have evolved to either lose their stomachs or reduce gastric acidity, minimizing the formation of calcium phosphate precipitates in the intestine.

**Keywords:** bone calcification; external fertilization; phosphorus absorption; reproduction; stomach loss

**Key Contribution:** This paper presents the likely causes of the evolutionary loss of acid-secreting stomachs and endoskeletal ossification in vertebrates.



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## 1. Introduction

In the 18th century, the Italian biologist Lazzaro Spallanzani studied a wide variety of animal species' digestive mechanisms with an emphasis on the functions of stomachs [1]. He demonstrated that gastric fluid plays a crucial role in preventing food spoilage in the stomach. In accord with this, the acid-secreting stomach first evolved in gnathostomes, which possessed strong jaws to prey on larger organisms. Spallanzani exemplified serpents, as they require several days to digest large prey. He also confirmed that gastric fluid could restore putrefied food—a common occurrence in the wild—highlighting its significance, particularly for carrion feeders like vultures, jackals, and hyenas.

Spallanzani found that gastric fluid possesses a strong solvent property, enabling solubilization of large prey in the stomach, while sending smaller amounts of chyme to the intestine for absorption. He identified the presence of marine acid, now known as hydrochloric acid, in the gastric fluid. Furthermore, he noted the absence of a stomach in the barbel, a species of fish, but did not delve into any details. Spallanzani's findings were complemented by his contemporaries, including Réaumur, Stevens, and Hunter, who made related contributions to the understanding of gastric physiology [2]. Currently, several lineages of fish are known to have lost their stomachs during the process of evolution (known as agastrics). Agastric species, therefore, have sacrificed all these apparently beneficial functions of the stomach. But why?

A critical investigation into the causes of stomach loss may require careful examination of the physiological and ecological differences among various animal species with varying levels of gastric acidity (not just agastric vs. monogastric). Although the driving causes of stomach loss have been extensively discussed by researchers from various backgrounds, the physiological advantages of stomach loss remain largely unknown [3–5]. However, to the best of the author's knowledge, this issue has never been analyzed from a phosphorus (P) perspective. In this context, I propose an alternative hypothesis based on P: Acid secretion was not only a futile effort but even detrimental to some ancient gnathostomes that eventually evolved into agastric species.

## 2. Stomach for Want of P?

Phosphorus is an essential element with numerous critical functions in the body [6]. However, unlike calcium (Ca) and other inorganic elements, P is scarcely present in ambient water, which means aquatic animals like fish must obtain it from their diets [7,8]. Invertebrates, including most protostome and deuterostome species, have relatively low P requirements due to their lower bodily P content. In contrast, vertebrates, including fish, have significantly higher P needs, primarily because their skeletal systems are composed of Ca phosphates. Indeed, fish are the most P-rich organisms in aquatic ecosystems [9].

To efficiently absorb dietary P, gnathostomes may evolve a stomach that secretes acid to dissolve Ca phosphate in vertebrate prey. However, this hypothesis may not hold true because many prey organisms contain considerable amounts of non-apatite P (i.e., P not bound to Ca). Invertebrate prey, like mollusks, crustaceans, and zooplankton, typically contain 0.8–0.9% non-apatite P per dry mass [9–11]. These levels of non-apatite P are higher than the dietary P requirements of most fish species (~0.6% per dry diet [12]), meaning that predatory fish can absorb sufficient P from prey without the need for acid secretion or dissolving bone-P (apatite-P).

For example, some cyprinids (agastric) are piscivorous and capable of absorbing only non-apatite P in baitfish [13,14]. The whole-body Ca content of baitfish is approximately 0.5% [10,11]. Given the molar ratio of Ca to P in hydroxyapatite (5/3), the mass ratio is about 200/93, yielding a bone-P content of around 0.23%. Since the total body P content of fish is around 0.45%, the non-apatite P is approximately 0.22% (wet basis). With an average water content of 75%, this equates to about 0.87% non-apatite P on a dry weight basis, which is well above dietary requirements. During the post-larval to early juvenile stages, when fish grow rapidly, they are most vulnerable to P deficiency [12]. However, during this period, fish typically consume zooplankton, which are rich in non-apatite P. Thus, even agastric fish can meet their P needs in their natural habitats.

An alternative explanation for acid-secreting stomachs may be that they evolved not primarily for P absorption but to dissolve bones and shells, preventing large fragments from obstructing the intestine or injuring enterocytes. This would be crucial for carnivores that swallow prey whole. However, piscivorous cyprinids and other agastric fish use pharyngeal teeth or analogous organs to masticate bones and shells, making acid secretion less necessary. In species without pharyngeal teeth, such as salmonids, acid secretion may still play a critical role in solubilizing prey bones. However, is this the only reason for the evolution of an acid-secreting stomach?

The acid-secreting stomach first appeared in elasmobranch fish [15]. In these cartilaginous fish, the stomach is highly acidic, with a 12-h postcibal pH as low as 2 [16]. Why do these primitive stomachs secrete strong acids that are stronger than those of most teleosts and comparable to mammals? While chondrichthyans may not need large amounts of P for their less calcified endoskeletons, they may require P for their teeth and placoid scales. Sharks, for instance, continuously replace their teeth at a replacement rate of 10–20 days

per row, depending on the species [17]. Since the primary constituents of teeth, dentin, and enamel are Ca phosphates, the P demand for tooth regeneration could be significant. Additionally, the elasmobranchs swallow their prey whole without mastication, and their stomach retention time is considerably long [18,19]. Hence, strong gastric acid enables piscivorous sharks to dissolve large prey, preserve them in the stomach without putrefaction, and efficiently absorb P.

Gastric acid dissolves hydroxyapatite-P in vertebrate prey, which is subsequently absorbed passively in the anterior intestine, including the pyloric ceca [20]. Hence, dietary acidification significantly enhances P absorption in fish meal-based aquaculture feed [21]. Nonetheless, any excess P above the fish's requirement will be excreted in urine. Considering that most prey organisms contain non-apatite P (bioavailable P) in amounts above the dietary requirement, dissolving apatite-P may not provide a meritorious effect. Therefore, the role of gastric acid secretion may not primarily be for P acquisition.

### 3. Is Stomach Necessary?

The stomach has various functions, such as food storage at low pH, acid disinfection of microbial contaminants, secretion of intrinsic factor (vitamin B<sub>12</sub>-binding protein), and partial digestion [22]. However, these functions might not be essential for survival, as gastrectomy in humans, for example, is not life-threatening. In species like carp, intrinsic factors may be supplied by microbial activity in their long, coiled intestines, while their constant nibbling and feeding habits eliminate the need for food storage.

Some agastrics (e.g., grass carp, silver carp, and scarids) are herbivorous or phytoplankton feeders. Agastric herbivores digest their food through trituration using their well-developed pharyngeal teeth or grinding mills. Mulletts have a stomach with weak acidity but compensate with a well-developed gizzard to grind leafy materials [23]. In contrast, cichlids such as tilapias possess a stomach, and their strong gastric acidity enables them to lyse algal cell walls [24]. Apparently, the stomach is dispensable when the trituration apparatus is present.

Carp, gobies, parrotfishes, and many other fish families or genera independently lost the acid-secreting stomach [5]. What is common among these agastric species is their benthic feeding habits, where they prey on bivalves, gastropods, crustaceans, and other shellfish high in Ca carbonate (CaCO<sub>3</sub>). As fish absorb sufficient Ca from the water via gills [25], dietary CaCO<sub>3</sub> is nutritionally useless for fish, except in very low Ca environments, which is rare even in freshwater habitats [8]. Furthermore, dietary CaCO<sub>3</sub> neutralizes gastric acid, rendering acid secretion futile. In fact, CaCO<sub>3</sub> is a common antacid in humans, used to buffer or neutralize gastric acid, providing relief from indigestion and heartburn [26,27]. Consequently, secreting gastric acid while consuming CaCO<sub>3</sub>-rich prey becomes largely a wasted effort [3]. By abandoning the energetically costly process of gastric acid secretion, carp and other agastric species could redirect energy toward other physiological needs, such as growth and reproduction [28,29]. This is certainly a compelling explanation. However, there may be additional, even more plausible, reasons for stomach loss among these species.

### 4. Alternative Perspective: Bioavailability of Dietary P

In various animals, including fish, the intestinal absorption of dietary inorganic phosphate (Pi) is heavily influenced by the presence of Ca and other cations in the same diet. These minerals can combine with Pi in the intestine to form insoluble salts, such as Ca phosphates, which reduce P absorption. Crushed shells processed by fish with pharyngeal teeth or analogous organs remain inert in the gastrointestinal tract unless solubilized by stomach acid [14]. As early as 1899, Paton et al. [30] wrote, "Lime salts are thought to form insoluble compounds with phosphoric acid in the intestine, thus preventing its absorption".

Therefore, acid secretion could be detrimental when  $\text{CaCO}_3$ -rich prey are involved, as solubilized Ca could reduce P absorption. By eliminating the stomach, agastric species have optimized P absorption from their  $\text{CaCO}_3$ -rich diets (Table 1).

**Table 1.** Absorption of calcium and phosphorus from different types of prey by animals with different gastric acidity.

| Prey                     | Absorption <sup>4</sup> | No Acid Secretion <sup>5</sup><br>(pH 6–7) | Weakly Acidic Stomach <sup>6</sup><br>(pH 3–4) | Highly Acidic Stomach <sup>7</sup><br>(pH < 2) |
|--------------------------|-------------------------|--------------------------------------------|------------------------------------------------|------------------------------------------------|
| Shellfishes <sup>1</sup> | Ca                      | ±                                          | +                                              | ++                                             |
|                          | P                       | +                                          | +                                              | ±                                              |
| Fishes <sup>2</sup>      | Ca                      | 0                                          | +                                              | ++                                             |
|                          | P                       | +                                          | +                                              | ++                                             |
| Low Ca <sup>3</sup>      | Ca                      | +                                          | +                                              | +                                              |
|                          | P                       | +                                          | +                                              | +                                              |

<sup>1</sup> Calcium carbonate  $\text{CaCO}_3$ -rich prey (e.g., mollusks, crustaceans, echinoderms, and corals). <sup>2</sup> Calcium phosphate or hydroxyapatite  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ -rich prey (i.e., vertebrates). <sup>3</sup> Low-calcium prey (e.g., insects, annelids, and plants). <sup>4</sup> Absorption efficiency of Ca and P by three types of animal species (no acid, weakly acidic, and highly acidic stomachs). <sup>5</sup> Fishes (e.g., cyprinids, belonids, cyprinodontids, tetraodontids, labrids, dipnoids, holocephalans, agnathans) and animals (monotremes, larval stomach of amphibians, and many herbivores, including colobine monkeys with no functional stomach). <sup>6</sup> Most actinopterygian fishes. <sup>7</sup> Birds, mammals (carnivorous and omnivorous), and some fishes (elasmobranchs, tilapia, and clariid catfish). Symbols: 0 (no absorption), ± (slight absorption); + (low absorption; the requirement level), ++ (high absorption).

In fish, Phillips et al. [31] conducted a landmark study in which brook trout were fed synthetic diets containing  $^{32}\text{P}$  and varying levels of Ca. When the diet lacked Ca, the fish absorbed all of the dietary P within 24 h. However, at a Ca/P ratio of 1/1, about 15% of the dietary P remained unabsorbed, and at a Ca/P ratio of 4/1, 40% of the dietary P remained unabsorbed after 24 h. In subsequent studies, Phillips et al. [32,33] found that increasing dietary Ca progressively reduced P utilization, with the highest P retention occurring at a Ca/P ratio of 0/1. Similarly, Nakamura [34] fed common carp semi-purified diets containing varying levels of Ca (0.1–2.6%) while maintaining a constant P level (0.64%). The sources of Ca and P (Ca lactate and  $\text{KH}_2\text{PO}_4$ ) were both highly soluble. The highest P absorption (98%) was observed with the lowest Ca content (0.1%), and P absorption decreased as dietary Ca increased.

In humans, hyperphosphatemia is common in individuals with chronic kidney disease (CKD), leading to vascular calcification and an increased risk of mortality. Consequently, lowering the serum Pi levels is crucial in these patients. To achieve this, various compounds known as P binders are used to reduce intestinal Pi absorption. Among the most commonly used are Ca carbonate, Ca acetate, and Ca lactate [35]. Most importantly, Ca carbonate requires strong gastric acidity to be effective, whereas more soluble Ca acetate and lactate work across a broader range of pH levels [36].

In pufferfish, which lack a functional stomach, Laining et al. [37] found that adding 1.5%  $\text{CaCO}_3$  to a dry diet did not reduce P absorption, although addition of 3.0% caused a slight reduction. Since  $\text{CaCO}_3$  is most soluble at pH 1–3, its dissolution in the stomach is necessary for effective P binding [38]. The absence of a negative effect on P absorption in pufferfish may be due to the unaltered passage of  $\text{CaCO}_3$  through the stomach, which prevents reactions between Ca and P in the intestine. This suggests that the interaction between Ca and P depends on the chemical form of Ca and the presence of gastric acidity [39]. Soluble Ca compounds are generally more effective at preventing intestinal P absorption than  $\text{CaCO}_3$  [40].

Of course, not all agastrics are benthic or shellfish feeders (e.g., grass carp, silver carp). These species may have secondarily evolved from a common ancestor similar to the

common carp. In addition to cyprinids, other fish species like pufferfish (Tetraodontidae), parrotfish (Scaridae), and wrasses (Labridae) also lack a functional stomach. These fish feed on  $\text{CaCO}_3$ -rich benthic organisms, such as clams, snails, and crustaceans, utilizing well-developed incisors or pharyngeal teeth. Similarly, beloniforms, which are stomachless carnivores, spawn in drifting algae near the ocean surface, where their larval and juvenile stages—most susceptible to P deficiency—feed on  $\text{CaCO}_3$ -rich crustaceans (e.g., amphipods, zoea, megalopa) found in the algae, also using pharyngeal teeth. The dipnoians and holocephalians, both of which are durophagous agastrics, use tooth plates to crush the hard shells of their prey [41,42].

The Senegal sole, a marine flatfish, does possess a stomach but secretes very little acid, maintaining a nearly neutral gastric pH even after feeding [43]. Similar to other agastric species, it feeds on  $\text{CaCO}_3$ -rich benthic invertebrates, including clams and crustaceans. For these species, evolutionary loss of the stomach or its functions is likely a food-driven adaptation, reflecting selective pressures related to their diet.

The gastric pH of salmonids, particularly Atlantic salmon, is relatively high, ranging from pH 4.0 to 5.2 [44,45]. These and other salmonids feed on various crustacean prey in ocean habitats, which contributes to their pink coloration due to prey-derived astaxanthin. Crustaceans are rich in  $\text{CaCO}_3$ , which is only minimally soluble at pH 4. It seems that salmonids finely adjust their gastric pH to minimize  $\text{CaCO}_3$  solubilization while at the same time preventing spoilage of prey in the stomach.

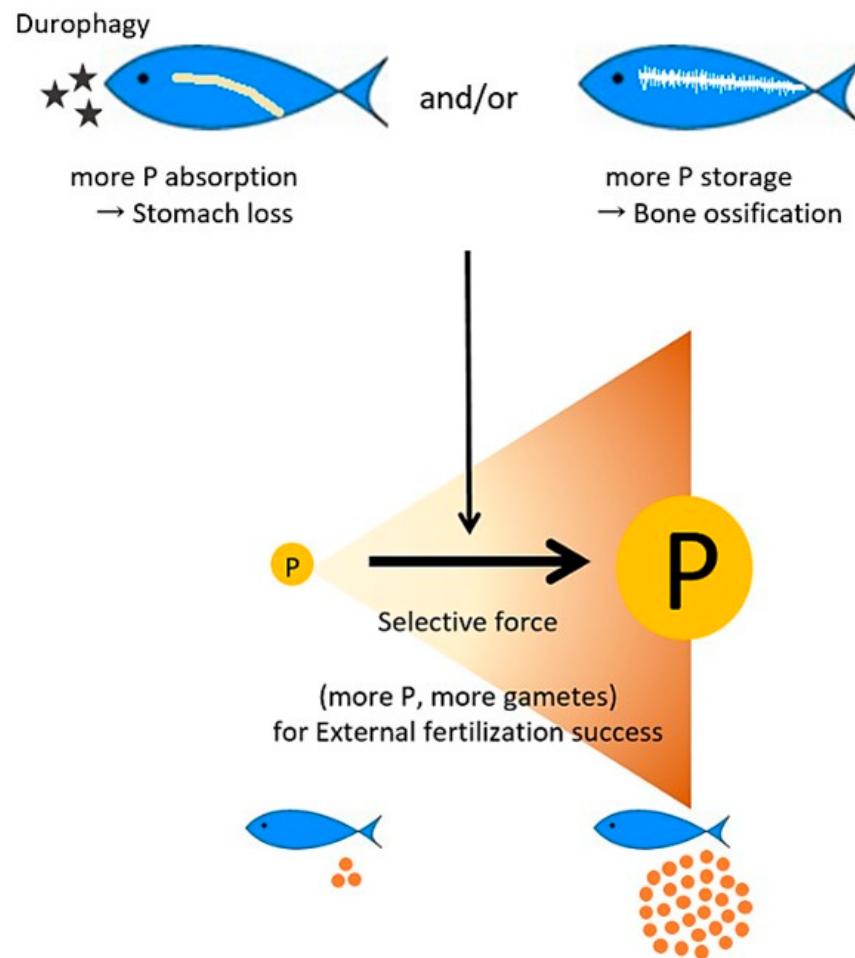
The loss of the stomach may have occurred independently across different teleost lineages, but the reason appears to be common: preventing Ca-P precipitation in the gut. This finding is supported by the fact that most, if not all, agastric species are durophagous. For many teleosts, acquiring large amounts of P from their diverse prey seems critical for reproductive success, given the high demand for P for external fertilization, as discussed below.

## 5. External Fertilization Elicited Vertebral Calcification

Unlike land animals and chondrichthyans, most teleosts rely on external fertilization. External fertilization is much less efficient than internal fertilization in terms of both fertilization rate and survival rate of offspring, as reasoned by Aristotle [46] and studied by numerous researchers in a wide variety of species [47]. Many teleosts, such as salmon, smelt, and eel, develop significantly large ovaries and testes as they mature, with gametes occupying a predominant portion of the intra-peritoneal cavity, compressing other internal organs, including the gastrointestinal tract. In general, larger ovaries and testes can enhance the success rate of external fertilization (Figure 1).

As gametes are particularly rich in P, large amounts of P are required for gametogenesis in both ovaries and testes, especially in the latter. For example, studies on maturing Biwa salmon *Oncorhynchus masou rhodurus* have shown a significant decrease in vertebral ash and P concentrations in males (discussed below), suggesting mobilization of bone-derived P for spermatogenesis [48]. It can be inferred, therefore, that the primary role of fish bones might be as a P reservoir for reproduction. In other words, ancient fish likely evolved calcified bones to store large amounts of P in the form of Ca phosphate (hydroxyapatite), providing the necessary resources for reproductive processes.

Early research by Meischer [49] and Paton [50] found that in salmon, P stored in muscles as simple phosphates is transferred to the ovaries and testes during reproduction, where it is converted into organic compounds. Although their studies did not measure P in hard tissues, later work by Milroy [51] on herring sought to identify the precursors of these organic P compounds that rapidly accumulate in the gonads during reproduction.



**Figure 1.** The large amounts of phosphorus (P) required for external fertilization drove vertebral ossification and, in durophagous fishes, the loss of the stomach. In most actinopterygians, the evolution of traits to enhance reproductive success created strong selective pressure to store abundant P in their bodies, optimizing gamete production for external fertilization. Selective advantages are also evident in adaptations, such as stomach loss or reduced gastric acidity, which improve P absorption from prey, particularly in durophagous fishes. In the figure, black stars represent  $\text{CaCO}_3$ -rich prey (e.g., mollusks, crustaceans, echinoderms, and corals) and orange dots indicate male and female gametes.

The P content of maturing ovaries or eggs is notably high due to the presence of phosphoproteins, while the P content in the testes is also significant because of the nucleic acids involved. Bruce [52] reported that maturing herring gonads contained between 0.37–0.38% P in females and 0.51–0.57% P in males. The author wrote, “the developing gonads make a heavy demand for phosphorus, the testes having relatively the higher requirement”.

Vinogradov [53] compiled extensive data on the mineral compositions available at that time. From his Table #295, he reported that the mineral content of fish roe (as a percentage of living matter) was as follows: sturgeon roe contained 0.13% Ca and 0.67% P; salmon roe had 0.10% Ca and 0.35% P; trout roe, 0.05% Ca and 0.33% P; pike roe, 0.04% Ca and 0.31% P; cod roe, 0.09% Ca and 0.31% P; and seabass roe, 0.02% Ca and 0.11% P. He noted that P levels in roe were significantly higher than Ca, remarking, “The P is highest in the roe, . . . In the roe, there is 5 to 10 times more P than Ca”. MEXT [10] similarly reported that cod roe contains 0.02% Ca and 0.39% P, while cod milt contains 0.01% Ca and 0.43% P. The high P content in eggs is likely due to the substantial forthcoming need for P during the prelarval stage, as it is essential for DNA synthesis and skeletal growth. During this period, fish can

absorb sufficient Ca from water [54]. The elevated P content in milt may be attributed to the high concentrations of nucleic acids and ATP in the sperm.

While Ca is abundant in the surrounding water, P can only be sourced from the diet. During gonadal maturation, however, anadromous salmon with a semelparous life history essentially stop feeding. Consequently, the high P content found in the growing ovary (i.e., vitellogenin and phosvitin) and testis (rich in DNA and ATP) must have originated from non-dietary sources. As P concentrations in water are extremely low and absorption capacity is minimal [11], the primary source of P for gonadal maturation is likely the body's own reserves, such as bones and scales, which serve as P storehouses. Indeed, significant bone resorption occurs during this period to supply the necessary P for spermatogenesis and oogenesis, as discussed below.

Tartrate-resistant acid phosphatase (TRAP) is a marker enzyme for osteoclast activity in bones and scales. Persson et al. [55] found that injecting juvenile rainbow trout (body weight 43 g) with estradiol-17 $\beta$  (E2) increased osteoclastic activity (measured by TRAP levels) and plasma Ca concentrations while reducing the Ca content in scales, indicating increased resorption. They suggested that during sexual maturation, rainbow trout protect their skeletons using scales as a Ca source. No mention was made of P. Similar results were reported by Persson et al. [56], and in subsequent studies [57,58], increased scale resorption with elevated TRAP and plasma Ca levels was observed in both male and female maturing trout. However, these authors attributed the increased resorption in river-ascending salmon and E2-injected trout to the increased need for Ca. Given the abundance of Ca in water and its efficient absorption, as well as the significantly lower Ca content in gonads compared to P, it is more plausible that bone and scale resorption during sexual maturation primarily serves to mobilize P for gonads. This has been experimentally confirmed by Yamada et al. [59], Witten and Hall [60], and others.

Earlier studies, such as those by Lopez et al. [61], also found that female eels undergoing sexual maturation exhibited bone decalcification (osteoclastic resorption) accompanied by elevated serum Ca and P levels. Kacem et al. [62] and Kacem and Meunier [63] observed significant reductions in vertebral ash content in Atlantic salmon during spawning migration, with similar degrees of demineralization reported for both male and female fish. These findings are consistent with those of other studies documenting reductions in vertebral or scale mineral content during sexual maturation in various fish species (reviewed by Kacem et al. [62]).

The primary function of calcified bones (and scales) in fish appears to be to serve as major P storehouses, supplying large quantities of P for gametogenesis. Additionally, hydroxyapatite bones support vigorous locomotive activities, such as anadromous migration. Ca phosphate endoskeletons, rather than Ca carbonate endoskeletons, allow fish to utilize more ATP during intense muscular activity, which locally produces lactic acid through anaerobic glycolysis. Ca phosphate is less soluble in acidic conditions compared to Ca carbonate [64–66]. Furthermore, bones likely play a crucial role in scale regeneration, as P is translocated from the bone to new scales to prepare for potential predatory attacks as quickly as possible [67].

Research suggests that male salmon may be more prone to P deficiency during maturation than females, which could explain why female salmon are responsible for creating spawning beds, or “redds”. Taguchi et al. [48] conducted an experiment with maturing Biwa salmon over 90 days, feeding them either a low P diet (0.36% available P content) or a commercial diet (0.49% available P content). The study found that fish on the low P diet exhibited significantly lower bone ash content in males (44%) compared to females (51%), while both sexes on the commercial diet maintained normal bone ash content (54% in males, 51% in females). In addition, the bone P content of fish on the low P diet was

lower in males (8.3%) than in females (9.4%). These findings imply that male fish require more P than females during gametogenesis, confirming the findings of Bruce [52]. The lower bone ash content observed in maturing male salmon may explain why female salmon are responsible for digging redds in the wild, a task that involves using their caudal fins to move river gravel. Male salmon with their demineralized bones may lack the physical strength to perform this task, compounded by reduced ATP levels associated with P deficiency, particularly during and after exercise (reviewed in Sugiura [11]).

During gametogenesis, osteoclast activity in the scales increases, resulting in demineralization of the scales. Estrogen receptors are present in the scales, bones, and intestines [68]. Estradiol (E2) is known to mobilize scale minerals through increased osteoclast activity while enhancing the absorption of minerals in the intestines [69]. Pinto et al. [70] demonstrated that seabass experiences significant increases in plasma Ca, P, and vitellogenin levels five days after receiving an E2 injection. Many researchers have attributed this scale turnover to the increased need for Ca during gonadal maturation [58,68,70–75]. However, it is more plausible that this process is driven by an increased demand for P rather than Ca.

In maturing tilapia, Urasa et al. [76] found no detectable mobilization of bone Ca and P during the ovarian cycle under normal conditions. However, when female tilapia were fed a P-deficient diet, bone P was mobilized. The P content of the eggs from these fish was only slightly lower than that of the control group, but the spawning interval increased from 18 days (control) to 27 days in P-deficient fish. Plasma Ca concentrations in the females correlated with the gonadosomatic index (GSI), indicating increased bone resorption to withdraw P as the fish matured.

Obviously, P deficiency has a detrimental effect on reproductive capacity, which in turn drives the process of natural selection, favoring the development of enhanced P absorption mechanisms. In many actinopterygians, this evolutionary adaptation might involve the complete abandonment of the stomach or a significant reduction in gastric acidity to optimize P absorption in the intestine and/or the development of P storage organs in the body (e.g., apatite bones, scales).

Vertebral calcification first appeared in some Devonian osteichthyans and is likely to enhance the efficiency of external fertilization, as proposed in this paper. However, as vertebrates transitioned from aquatic to terrestrial environments and from external to internal fertilization—which requires much less P for gametogenesis—the role of calcified bones underwent a dramatic shift (more discussion below).

## 6. What About Land Animals?

As Aristotle noted [46], species that rely on internal fertilization, such as most terrestrial animals and chondrichthyans, produce only a small quantity of male and female gametes. This means that for these species, the P requirements for gametogenesis are relatively low compared to species that rely on external fertilization that produce large amounts of gametes. Thus, the P hypothesis does not adequately explain stomach loss and skeletal ossification in terrestrial animals.

In terrestrial animals, the primary function of gastric acid secretion is likely to be Ca acquisition. Soft tissues like flesh and viscera contain little Ca, so carnivores rely on bones (Ca phosphate) and shells (Ca carbonate) as their main Ca sources, both of which require acid solubilization for absorption. Moreover, terrestrial animals have to invest more in skeletal P and Ca to support their bodies against gravity [77]. For instance, the whole-body Ca content of adult humans averages 1.6% (wet body basis [78]), 2–4 times higher than that of fish (0.4–0.7%). Fish that float in water do not require as much bone strength.

Land animals might therefore increase their gastric acidity to dissolve Ca compounds in their food by evolving parietal cells to replace less efficient oxyntopeptic cells, creating a

highly acidic stomach [13,15]. In addition, in birds that lay eggs with  $\text{CaCO}_3$  shells, strong gastric acid helps enhance Ca absorption from their prey. Of course, gastric acid sterilizes food microbes, making food storage possible. These functions highlight the benefits of acid-secreting stomachs in land animals. However, in some species (described below), these benefits may not be helpful, potentially reducing the necessity for a functional stomach.

The platypuses *Ornithorhynchus anatinus* forage on various species of aquatic insects, along with significant proportions of mollusks and crustaceans. Another group of monotremes, echidnas, feed on insects and annelid worms [79–81]. Notably, earthworms contain relatively high concentrations of Ca [82]. Their prey is fresh and easily digestible, reducing the need for gastric acid secretion.

Many herbivorous mammals, including leaf-eating monkeys, are known to have nearly neutral stomachs [83]. They feed primarily on fresh leaves, which contain few pathogenic bacteria compared to meat or fish, rendering gastric acid unnecessary for these animals. However, the natural diets of herbivores are often deficient in Ca and even more so in sodium. Therefore, how do they meet their mineral requirements? They may obtain various minerals not from the diet but from natural licks [84–86]. Additionally, terrestrial animals develop a specific appetite for these minerals when their dietary intake is insufficient [87]. Therefore, for herbivores, strong stomach acids may not offer significant benefits.

Consequently, even though mutations occurred in genes related to stomach function, these animals were not negatively selected for survival. In fact, such mutations may have had an overall positive effect, as the stomach is a double-edged sword. Gastric acid secretion is energetically costly, and maintaining the stomach imposes a significant metabolic burden [29]. Thus, the reduction or complete loss of stomach function could enhance a species' survival through natural selection. The disuse or loss of the stomach did not result in negative consequences, but could instead be advantageous by eliminating both its benefits and drawbacks.

Many anuran tadpoles have an underdeveloped stomach, known as a larval stomach, which does not secrete acid or pepsin. However, after metamorphosis, the stomach becomes fully functional [88]. Tadpoles are generally omnivorous, feeding on algae, small crustaceans, and mollusks. Producing acid to digest such  $\text{CaCO}_3$ -rich prey would reduce intestinal P absorption. In contrast, adult frogs need to obtain sufficient Ca from Ca-poor insects [89]. Therefore, they require an acidic stomach to digest and absorb Ca efficiently in terrestrial environments. In many anurans, skeletal ossification likely evolved in response to their terrestrial habitats and external fertilization, both of which may have exerted strong selective pressure for the development of well-ossified, robust bones, ultimately adapted to fully terrestrial environments.

Extant sarcopterygians (coelacanth and lungfish) have skeletons with minimal ossification; however, Devonian sarcopterygians, such as Eusthenopteron, had partially ossified skeletons [90–92], implying their dependency on bone-stored P for gametogenesis, at least partly. This may have marked the origin of vertebral calcification, enabling sarcopterygians to evolve into land-dwelling animals. Although some mammals, such as cetaceans and sirenians, transitioned to fully aquatic habitats, they did not lose their ossified bones. Instead, they adapted these bones for use as buoyancy-controlling organs [93,94]. The functions and roles of bones appear to be highly variable among animals adapted to different habitats.

## 7. External Fertilization Without Skeletal Ossification

The advantages of internal fertilization include minimal wastage of gametes, high fertilization rate, and high survival rate of offspring. Its key disadvantage is the relatively small number of offspring that can be produced, which reflects the differences between r- and K-selection strategies [95]. Aquatic animals that rely on internal fertilization, such as

chondrichthyans and coelacanth [92,96], require less P than externally fertilized species. Consequently, they did not develop highly ossified skeletons and, as a result, were unable to expand their habitats to terrestrial environments. Most gastropods, cephalopods, and arthropods primarily reproduce via internal fertilization or similar systems. They produce relatively small amounts of gametes, which reduces their P requirements. Consequently, they have not evolved P storage organs like hydroxyapatite skeletons.

In contrast, aquatic animals that rely on external fertilization require high levels of P for gametogenesis. The development of a P storage organ, such as ossified vertebrae, was therefore necessary or at least beneficial for these species. Nonetheless, some species that rely on external fertilization still do not ossify their skeletons. Then, how do they manage to achieve external fertilization successfully?

Extant dipnoids (lungfish), a sister group of tetrapods, lay many eggs and fertilize them externally [97]. However, fertilization takes place in enclosed spaces, such as nests or stagnant water, which reduces the number of gametes required and eliminates the need for a well-ossified skeleton. Being iteroparous [97], they likely feed during gametogenesis, further reducing the necessity for P reserves in their bodies. Similarly, agnathans lay a relatively small number of eggs multiple times or produce many small eggs. Their testes are small, and eggs fertilized in enclosed spaces require minimal gametes, leading to low P requirements for gametogenesis [98–100]. Primitive actinopterygians (e.g., chondrosteans and cladistians), which have less calcified bones, store P in structures like scutes and ganoid scales.

Echinoderms, despite lacking vertebrae, successfully achieve external fertilization. They have developed nutrient-storage cells known as nutritive phagocytes. These cells store and transfer nutrients to developing gametes; thus, their volume is inversely proportional to the volume of gametes in both males and females [101–103]. Apparently, among various externally fertilizing species, endoskeletal ossification was only one of the many paths in adaptive evolution strategies.

## 8. Conclusions

The present paper only addressed a single perspective (i.e., the P-perspective) using mostly a deductive mold. However, the scientific process, being inherently inductive, necessitates the integration of diverse perspectives—such as physiological, ecological, taxonomical, molecular, and microbial—to establish a more comprehensive and robust epistemological framework. Developing such an interdisciplinary understanding requires collective effort, as individual researchers are often highly specialized within their respective fields of expertise. Complex phenomena, such as stomach loss and vertebral calcification, represent significant scientific challenges that demand a more holistic, integrative, and methodologically rigorous approach to effectively address their underlying mechanisms.

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