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Skeletal deformities in wild and farmed cleaner fish species used in Atlantic salmon *Salmo salar* aquaculture

Per Gunnar Fjelldal[1] | Angelico Madaro[2] | Malthe Hvas[1] | Lars Helge Stien[1] |
Frode Oppedal[1] | Thomas WK Fraser[1]

[1]Matre Research Station, Institute of Marine Research, Matredal, Norway

[2]Institute of Marine Research, Bergen, Norway

Correspondence
Per Gunnar Fjelldal, Matre Research Station, Institute of Marine Research, Havforskningsinstituttet Matre, Matre 5, 5984 Matredal, Norway.
Email: pergf@hi.no

Abstract

As a first attempt to assess bone health in cleaner fish production, wild and cultured ballan wrasse *Labrus bergylta* and lumpfish *Cyclopterus lumpus* were examined by radiology. In *C. lumpus*, wild fish (57%) had more vertebra deformities (≥1 deformed vertebrae) than cultured fish (2–16%). One wild *C. lumpus* had lordosis and another was missing the tail fin. In *L. bergylta*, wild fish (11%) had fewer vertebra deformities than cultured individuals (78–91%). Among the cultured *L. bergylta*, 17–53% of the fish had severe vertebra deformities (≥6 deformed vertebrae) with two predominate sites of location, one between vertebra 4 and 10 (S1) in the trunk, and one between 19 and 26 (S2) in the tail. Fusions dominated S1, while compressions dominated S2. Although wild *L. bergylta* had a low vertebra deformity level, 83% had calluses and 14% had fractures in haemal/neural spines and/or ribs. The site-specific appearance and pathology of fracture and callus in wild *L. bergylta* suggests these are induced by chronic mechanical stress, and a possible pathogenesis for fish hyperostosis is presented based on this notion. In conclusion, good bone health was documented in cultured *C. lumpus*, but cultured *L. bergylta* suffered poor bone health. How this affects survival, growth, swimming abilities and welfare in cultured wrasse should be further investigated.

Significance Statement: Skeletal deformities were studied in ballan wrasse and lumpfish of both wild and cultured origin for the first time to identify potential welfare issues when deploying them as cleaner fish in salmon sea cages. While cultured lumpfish showed good bone health, cultured wrasse had a high occurrence of vertebra deformities, which is expected to impact lice eating efficiency and animal welfare negatively. These deformities are most likely induced early in development.

KEYWORDS
Ballan wrasse *Labrus bergylta*, fish welfare, lumpfish *Cylopterus lumpus*, osteomas, salmon louse *Lepeophtheirus salmonis*, vertebral column

1 | INTRODUCTION

Infestation by the sea lice *Lepeophtheirus salmonis* K. 1837 and to lesser extent *Caligus elongatus* N. 1832 are major challenges in sea cage based aquaculture of salmonids in the northern hemisphere while species such as *Caligus teres* W. 1905 and *C. rogercresseyi* B. & B. 2000 pester the southern hemisphere (Costello, 2009; Abolofia *et al.*, 2017; Brooker *et al.*, 2018). To control sea lice levels on farmed *Salmo salar*, chemical, mechanical and thermal treatments are currently in use (Overton *et al.*, 2018), while technological solutions to minimize risks of infestations are emerging (Stien *et al.*, 2018). However, as an alternative to other delousing methods, deployment of cleaner fish (Bjordal, 1990) in sea cages is growing in popularity as they have been found to efficiently remove *L. salmonis* from *S. salar* (Imsland *et al.*, 2018a; Leclercq *et al.*, 2014; Liu & Bjelland, 2014). The number of cleaner fish deployed has therefore increased drastically in recent years from fewer than 2 million fish in 2008 to more than 50 million fish in 2017 in Norway alone (Norwegian Directorate of Fisheries, 2018). Ballan wrasse *Labrus bergylta* A. 1767 (Skiftesvik *et al.*, 2013) and lumpfish *Cyclopterus lumpus* L. 1758 (Powell *et al.*, 2018) are the most commonly used species. Initially, only wild wrasse species were deployed (Gonzalez & de Boer, 2017; Treasurer, 2002), but cultured *L. bergylta* (Skiftesvik *et al.*, 2013) and *C. lumpus* (Imsland *et al.*, 2014a) are now widely used.

When cleaner fish are used in Atlantic salmon sea-cage aquaculture, they must cope with strong tidal currents and hunt sea lice on the constantly swimming salmon. For this purpose, they rely on an anatomically functional vertebral column. Deformities in this structure may compromise swimming ability (Basaran *et al.*, 2007; Powell *et al.*, 2009). Since both *L. bergylta* and *C. lumpus* are new species in aquaculture (*e.g.*, Powell *et al.*, 2018), fundamental health aspects such as skeletal development and deformities have not yet been studied. Skeletal deformities are known to develop in both cultured and wild fish, but the occurrence is generally higher in cultured compared to wild fish (Boglione *et al.*, 2001; Fjelldal *et al.*, 2009a).

The teleost vertebral column is built up of amphicoelous (hourglass shaped) vertebrae that are separated by notochordal tissue, where trunkal vertebrae are rib bearing in contrast to caudal vertebrae that have haemal arches and spines (Ford, 1937). Both trunkal and caudal vertebra have neural arches and spines. The most common vertebra body deformities are compressions (Witten *et al.*, 2005), fusions and cases where two or more adjacent vertebra fuse and remodel in to a "normal" enlarged vertebra (Witten *et al.*, 2006). In compressed vertebrae, the prefect hourglass-shaped biconoid amphicoelous core is malformed, giving the vertebra an anterior–posterior compressed phenotype (Berg *et al.*, 2006; Witten *et al.*, 2005). Fusion normally occurs secondary to compression (Witten *et al.*, 2006). In cultured *S. salar*, reduced growth has been observed when more than 15% of their vertebra bodies are deformed (Hansen *et al.*, 2010). Growth performance is a key indicator of animal welfare (EFSA, 2008), and a deformity level that affects growth can therefore be considered severe. In addition to the deformities of the vertebral bodies, the whole vertebral column can curve, and lordosis, scoliosis and kyphosis

have all been reported in fish (Witten *et al.*, 2009). Moreover, dorso-ventral shifts or reduced intervertebral spaces may occur between normal adjacent vertebra (Witten *et al.*, 2009). Pathologies may also occur in ribs, haemal and neural spines, and pterigophores. The bone in these structures can swell to form large calcified calluses. This condition has been reported in several species and is termed fish hyperostosis (Smith-Vaniz *et al.*, 1995). In addition, Fjelldal *et al.* (2018) reported fractures in neural and haemal spines of Atlantic cod *Gadus morhua* L. 1758.

The purpose of the present study was to assess the occurrence, severity and types of pathologies in the vertebral column of *L. bergylta* and *C. lumpus*, the two most commonly used cleaner fish in salmon aquaculture. This was achieved by radiological examination. Fish from both cultured and wild origins were analysed to allow us to define an acceptable baseline of vertebral column deformity.

2 | MATERIAL AND METHODS

All experiments were conducted in accordance with the laws and regulations of the Norwegian Regulation on Animal Experimentation 1996.

2.1 | Fish material

The number of individuals, size and origin for the different fish groups are summarized in Tables 1 and 2. Wild *L. bergylta* (Ballan Wild) and *C. lumpus* (Lump Wild) were purchased from a local fisherman. The fish were caught by standing nets at 20–50 m depth in Masfjorden, Norway, and kept frozen until defrosting and radiology.

Cultured *L. bergylta* were randomly sampled from a stock produced at the Austevoll Research Station, Institute of Marine Research (Ballan IMR). These had been raised from brood stock at the same facility. Three different groups of cultured *L. bergylta* were also collected from one commercial producer. These were transported to IMR from the commercial producer MOWI Norway, cleaner fish department, sites Rong and Sykkulven. One commercially cultured *L. bergylta* group was kept in holding tanks at the IMR Matre Research Station for 4–16 weeks before sampling (Ballan Com 1). The two other commercially cultured *L. bergylta* groups were sampled at transfer to sea cages at the Matre Research Station (Ballan Com 2A and Ballan Com 3A) and after 3 months in cages (Ballan Com 2B and Ballan Com 3B). Ballan Com 1, 2 and 3, and Ballan IMR were all from different year classes. Cultured *C. lumpus* were collected from the Matre Research Station on three occasions: (i) *C. lumpus* sampled from indoor tanks (Lump IMR 1), (ii) *C. lumpus* from another production batch that were sampled before transfer to sea cages (Lump IMR 2A), and (iii) the same batch as (ii), but sampled after 3 months in sea cages (Lump IMR 2B). The IMR *C. lumpus* had been raised from brood stock onwards at the IMR Austevoll Research Station before transfer to the Matre Research Station. Cultured *C. lumpus* were also collected from three different commercial sea cage sites: Lump Com 1, Lump Com

TABLE 1 Prevalence (%) of different types of vertebral column deformities in wild and cultured ballan wrasse

Group	N	Length (mm)	≥1 def v (%)	≥6 def v (%)	C v (%)	F&C v (%)	R v (%)	Other (%)	≥1 callus (%)	≥1 swollen (%)	≥1 fracture (%)	≥1 deviation (%)
Ballan Wild	92	387 (230–460)	11	1	33.3	13.3	53.3	0.0	83	60	14	35
Ballan IMR	51	79 (50–102)	78	37	47.2	21.0	8.9	22.2	2	0	0	0
Ballan Com 1	45	250 (226–286)	89	53	70.4	15.0	1.5	13.1	47	27	22	0
Ballan Com 2A	22	106 (77–130)	91	41	77.6	17.8	1.9	2.8	0	0	0	0
Ballan Com 2B	173	133 (89–160)	81	34	77.2	14.2	4.1	4.5	1	0	0	1
Ballan Com 3A	12	172 (164–183)	83	17	47.1	39.2	11.8	2.0	8	0	0	0
Ballan Com 3B	68	181 (156–216)	82	38	60.6	23.7	10.6	5.0	10	4	1	0

Note. N, number of individuals examined. Length numbers in brackets are minimum and maximum lengths. def v, deformed vertebrae; C v, compressed vertebrae; F&C v, fused and compressed vertebrae; R v, remodelled vertebrae. Other includes vertically shifted vertebrae and vertebrae with decreased intervertebral space. The four latter categories represent the percentage of individuals with ≥1 callused, swollen or fractured neural/haemal spine and/or rib, and with different types of deviations in the ribs. '≥1 def v', '≥6 def v', '≥1 callus', '≥1 swollen', '≥1 fracture' and '≥1 deviation' are percentages of individuals with each condition. 'C v', 'F&C v' and 'R v' are percentages of deformed vertebrae with each condition.

TABLE 2 Prevalence (%) of different types of vertebral column deformities in wild and cultured lumpfish

Group	N	Length (mm)	≥1 def v (%)	≥5 def v (%)	C v (%)	F&C v (%)	R v (%)	VS v (%)	RIS (%)	EL v (%)
Lump Wild	30	400(290–450)	57	23	13	27	21	23	16	0
Lump IMR 1	80	199(140–244)	16	3	29	18	50	0	3	0
Lump IMR 2A	129	122 (99–139)	2	2	0	0	0	0	71	29
Lump IMR 2B	105	130 (92–164)	2	0	0	0	0	33	67	0
Lump Com 1	47	107 (68–142)	4	0	67	0	0	33	0	0
Lump Com 2	41	110 (86–138)	12	0	21	58	21	0	0	0
Lump Com 3	36	126 (97–156)	11	0	38	0	50	12	0	0

Note. N, number of individuals examined. Length numbers in brackets are minimum and maximum lengths. def v, deformed vertebrae; C v, compressed vertebrae; F&C v, fused and compressed vertebrae; R v, remodelled vertebrae; VS v, vertically shifted vertebrae; RIS, reduced intervertebral space; EL, elongated vertebrae. '≥1 def v' and '≥5 def v' are percentages of individuals with each condition. 'C v', 'F&C v', 'R v', 'VS v' and 'RIS' are percentages of deformed vertebrae with each condition.

2 and Lump Com 3. These were all from different *C. lumpus* production sites. Lump IMR 1 was from a different year class than Lump IMR 2, and Lump Com 1, 2 and 3.

2.2 | Radiology

Fish were radiographed with a Direct Radiology System (Canon CXDI-410C Wireless, CANON INC., Kawasaki, Japan) using a portable X-ray unit (Portable X-ray Unit Hiray Plus, Model Porta 100 HF, JOB Corporation, Yokohama, Japan) at 88 cm distance with 40 kV and 10 mAs. *L. bergylta* and *C. lumpus* were first radiographed as whole fish, then the *C. lumpus* were filleted for a second round of radiography to get better radiographs of their vertebral columns (*C. lumpus* skin contains spines that are relatively radiodense and obstruct the vertebra). Each fish was evaluated for different types of vertebra deformities (Witten *et al.*, 2009) and type and location were recorded. The deformities were categorized into six main categories: compression (type 2, 3, 4, 5; Witten *et al.*, 2009), fusion (6, 8), remodelling (type 7), vertical shift (type 17), reduced intervertebral space (type 1) and lordosis (type 14). Additionally, the pterigophores, ribs, and neural and haemal

arches and spines were assessed for fractures and calluses (Fjelldal *et al.*, 2018).

2.3 | Calculations

For the percentage of deformed fish within a population, we present data on those with ≥1 deformed vertebra in both species, and we considered those with ≥5 and ≥6 to be more severely deformed fish in *C. lumpus* and *L. bergylta*, respectively, as previous work in *S. salar* demonstrated that individual fish with ≥15% radiologically deformed vertebra show a decrease in growth (Hansen *et al.*, 2010). In the present material, *L. bergylta* had 36 or 37 vertebrae and *C. lumpus* 27 or 28.

3 | RESULTS

3.1 | *L. bergylta*

The prevalence of vertebra pathologies in wild *L. bergylta* was 11% with only 1% with ≥6 deformed vertebra (Table 1). The most

prominent pathology was two vertebra that had fused and remodelled into a single vertebra with a normal X shape (type 7 in Witten *et al.*, 2009; Table 1 and Figure 1a). In contrast, the prevalence of calluses was 83% (Table 1) and located in three regions: in the neural spines 19 to 26 (Figure 1c,g), in the haemal spines nos. 21 to 29 (Figure 1g) and along the ribs 5 to 15 (Figure 1b,g). Calluses on anal fin pterigophores and tail fin lepidotrichs were also observed (Figure 1a). Up to four calluses could be observed on one neural spine (Figure 1d). Calluses had one of two phenotypes, either those with reduced radiodensity in the centre (Figure 1d or those with a consistent radiodensity throughout the entire structure (Figure 1e). Some haemal and neural spines were entirely swollen throughout, from the base to the tip. This "swollen" spine phenotype (Figure 1c) co-occurred in the same regions as the calluses. We observed fractures in both the ribs and the neural spines, although these occurred sporadically between vertebra 2 and 22 (Figure 1b). Finally, wild *L. bergylta* often had ribs that deviated from relatively straight structures to become curled and/or show prominent wiggles towards the tip (Figure 1b). Some ribs also lacked radiodensity within certain regions (Figure 1b). Abnormalities in the tail fin were readily observed, but not quantified

(Figure 1a). Of note, wild fish often had large calciferous masses passing through the intestinal system (Figure 1f).

In cultured *L. bergylta*, the prevalence of fish with ≥1 deformed vertebra was 7- to 8-fold higher than in wild *L. bergylta*, whereas the prevalence of severely affected fish with ≥6 deformed vertebra was 17- to 58-fold higher (Table 1). Vertebra deformities predominantly consisted of compressed and/or fused vertebra, whereas remodelled vertebra had a low prevalence (Table 1). The predominate regions for vertebra deformities in cultured *L. bergylta* were between vertebrae 4 and 10, and between 19 and 26 (Figure 2d). Fusions in which the inflicted vertebra were not remodelled with a typical X shape (types 6 and 8 in Witten *et al.*, 2009) dominated the cranial deformity region (Figure 2a,b,d), whereas vertebra with one-sided compressions, internal dorsal or ventral shifts, or homogenous compression, or those vertebra that were vertically shifted dominated the caudal deformity region (Figure 2a,c,d). Calluses, swelling or fractures in the spines or ribs were generally less prevalent than in wild fish, and only one farmed fish showed curled or wiggly ribs (Table 1). Other notable observations include one fish with a neural spine fused to a dorsal fin pterigophore (Figure 2e), one fish with a split neural spine, giving the spine a "pitchfork" phenotype (Figure 2f),
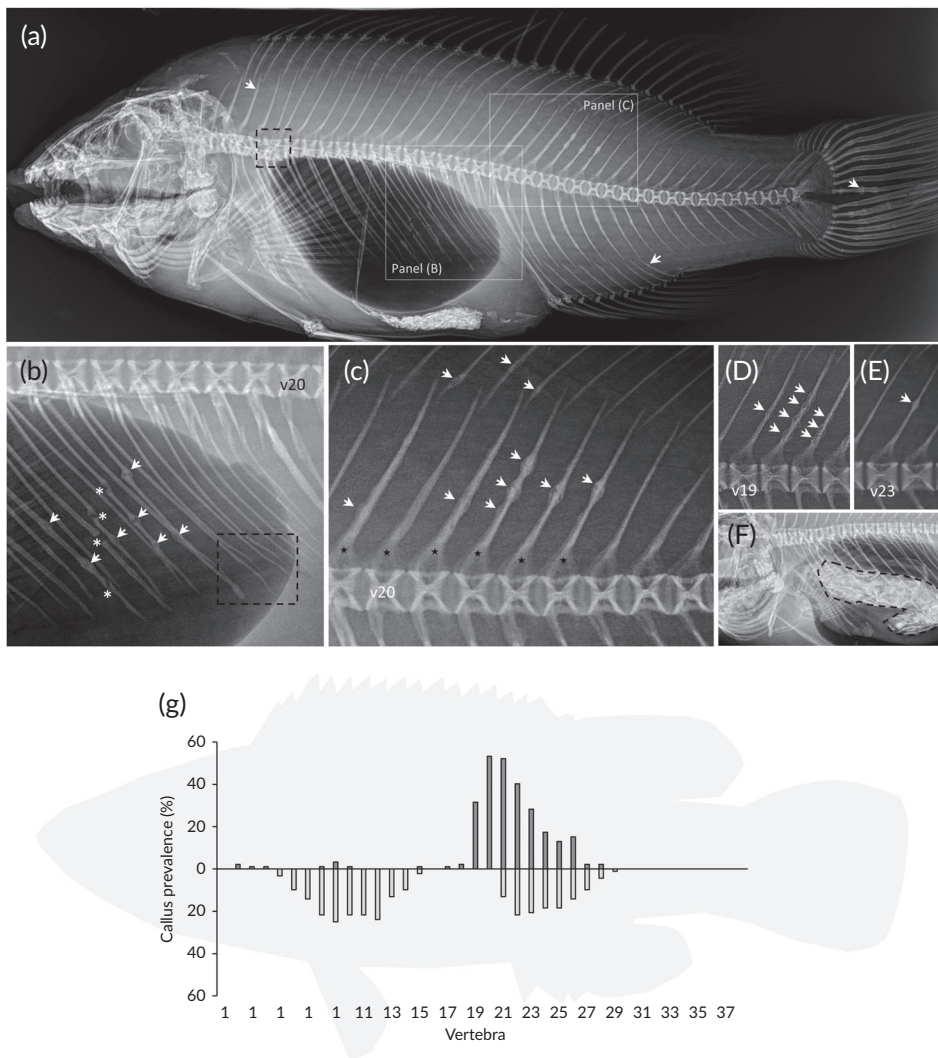

FIGURE 1 Skeletal pathologies in wild ballan wrasse, lateral radiographs. (a) Whole skeleton. White arrowheads indicate a callus in neural spine 3, a callus in an anal fin pterigophore and a callus within a tail fin lepidotrich. Note that not all calluses are highlighted for clarity. Within the dashed black outline, vertebrae 5 and 6 have fused and remodelled to form a single vertebra (type 7 in Witten *et al.*, 2009). (b) White asterisks indicate fractures and the dashed black outline highlights "wiggly" ribs, some are curled at the tip. White arrowheads indicate a callus. (c) White arrowheads indicate the numerous calluses observed in neural spines and pterigophores, whereas black stars highlight "swollen" neural spines. (d) White arrowheads indicate neural spine calluses lacking consistent radiodensity. (e) The white arrowhead indicates a neural spine callus with relatively consistent radiodensity. (f) The dashed black outline indicates a large calciferous mass within the intestinal system. (g) The prevalence of neural/haemal spines or ribs with callus formations within the entire population
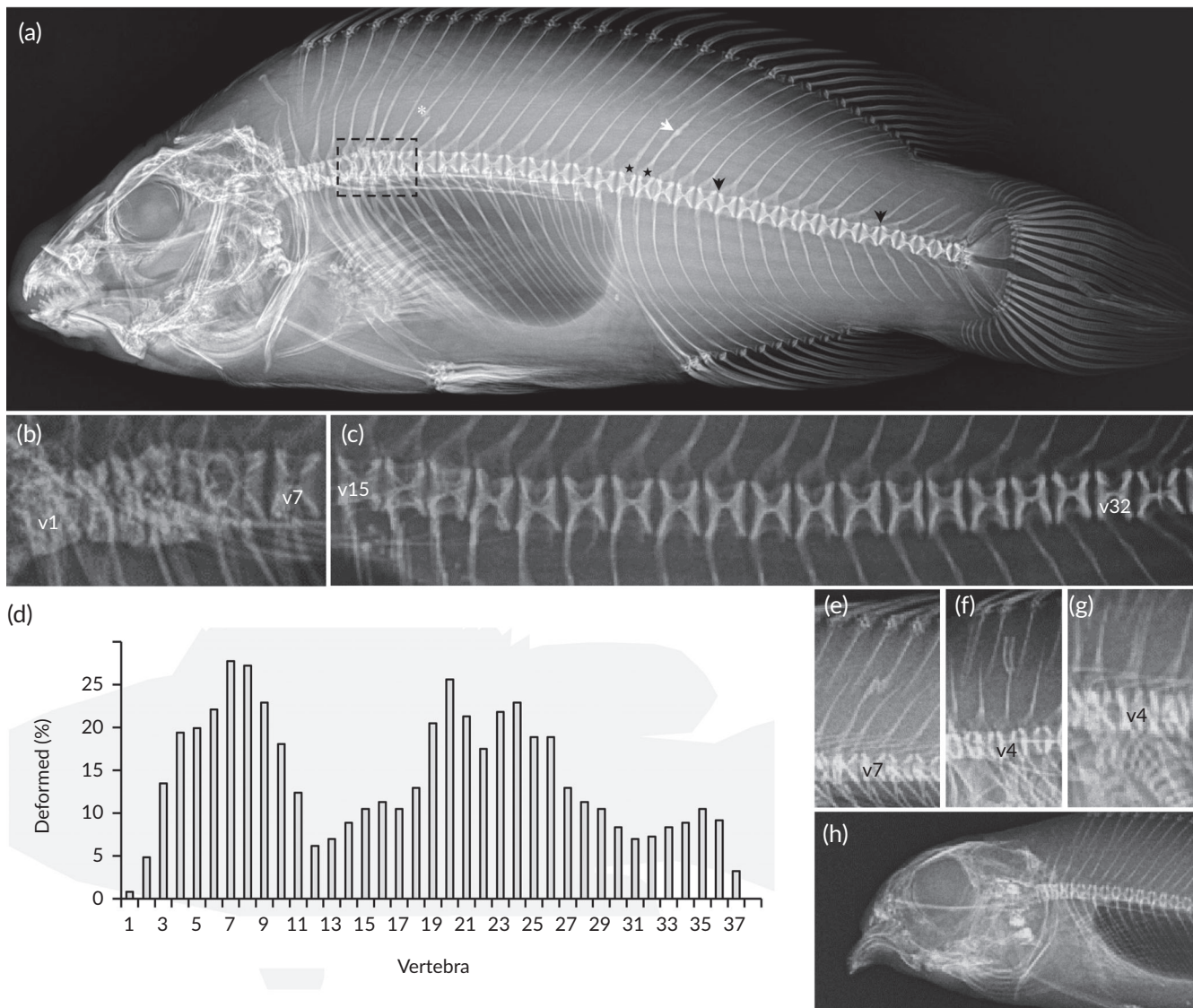
FIGURE 2 Skeletal pathologies in farmed ballan wrasse, lateral radiographs. (a) Whole skeleton. The white arrowhead indicates a "swollen" neural spine with calluses. The white asterisk represents a fracture within a neural spine. The dashed black outline indicates a fusion centre with compressed and fused vertebra (type 8 in Witten *et al.*, 2009). The black arrowheads indicate vertical shifts between adjacent vertebrae (type 17 in Witten *et al.*, 2009). The black stars show vertebrae with one-sided compression (type 5 in Witten *et al.*, 2009). (b) Fusion centre with compressed and fused vertebrae in the cranial trunk. (c) Multiple compressed vertebrae along the tail region of the vertebral column. Note also that vertebrae 33 and 34 have fused and remodelled into a single vertebra. (d) The prevalence of vertebra deformities along the vertebral column in farmed wrasse. The data were pooled for all populations of farmed fish. (e) A neural spine fused with a pterigophore. (f) A neural spine that is split towards the end, resulting in a "pitchfork" phenotype. (g) A vertebra with two neural spines, but only one pair of ribs. (h) Lower jaw deformity

one fish with one vertebra that had two neural spines, but only one pair of ribs (Figure 2g), one fish with a lower jaw deformity (Figure 2h), and one fish with a short opercula (not shown).

3.2 | *C. lumpus*

The prevalence of wild *C. lumpus* with vertebra deformities was 57%, with 23% having ≥6 deformed vertebrae (Table 2). The most prominent deformity types (Table 2) were compressed and fused vertebra (Figure 3b), vertically shifted vertebra (Figure 3c), and two/three

vertebra fused and remodelled into one (Figure 3d). One wild fish had lordosis, but with no external phenotype (Figure 3b). Vertebra deformities were most apparent around vertebra 10–11, but there was an even distribution of deformities along the vertebral column (Figure 3f). Other observations include one wild fish missing its entire tail and ural part of the vertebral column (Figure 3e), whereas another was missing half the tail fin (not shown). In both cases, the skin had completely healed, suggesting the fish had lived for some time with these conditions.

Farmed *C. lumpus* had substantially lower prevalences of deformed fish compared to their wild counterparts, with a 4–11-fold decrease in those with ≥1 deformed vertebra (Table 2). In general, vertebra

compressions without fusion were the most common deformity type, along with vertebra that had fused and successfully remodelled (Table 2).

4 | DISCUSSION

There are several studies on behaviour (Imsland *et al.*, 2014b, 2018b; Leclercq *et al.*, 2018), growth and cataract formation (Imsland *et al.*, 2018c), temperature effects and swimming capabilities (Hvas *et al.*, 2018; Yuen *et al.*, 2019), stress physiology (Hvas *et al.*, 2018; Jorgensen *et al.*, 2017; Piccinetti *et al.*, 2017), and diseases (Alarcon *et al.*, 2016; Ruane *et al.*, 2018; Treasurer, 2012) in cleaner fish, but this is the first attempt to assess and compare the incidence of bone pathologies between wild and cultured *L. bergylta* and *C. lumpus*. Screening different production batches of cultured fish and wild fish showed low and high vertebra deformity rates in cultured *C. lumpus* and *L. bergylta*, respectively, while among wild fish, *C. lumpus* had more deformities compared to *L. bergylta*. Furthermore, to our surprise, wild *L. bergylta* had a high occurrence of neural/haemal spine and rib pathologies, and lack of tail fin and lordosis were recorded in wild *C. lumpus*. The present study presents baseline data on occurrence and type of vertebral column deformities in *L. bergylta* and *C. lumpus*.

4.1 | Wild *C. lumpus* can survive with severe vertebra deformities

In the presently studied wild fish, the *L. bergylta* had a vertebra deformity rate of 12% and *C. lumpus* 57%. Earlier records on vertebra deformity rate in wild fish have shown 3–43% in salmonids (Fraser *et al.*, 2014; Gill & Fisk, 1966; Sambraus *et al.*, 2014) and 6–33% in gadoids (Fjelldal *et al.*, 2009a; Jawad *et al.*, 2018; Wunder, 1971). Surveys on deformity rate in wild marine fish in Masfjorden, Norway, the area where the currently investigated wild *L. bergylta* and *C. lumpus* were collected, have shown 6% in *G. morhua* (Fjelldal *et al.*, 2009a) and 33% in haddock *Melanogrammus aeglefinus* L. 1758 (Jawad *et al.*, 2018). Of the analysed wild *C. lumpus*, 23% were categorized as having severe vertebra deformities. One fish had severe lordosis, while another lacked the entire tail fin and the ural region of the vertebral column, but the remaining tail was completely healed. This shows that *C. lumpus* can survive in nature with severe deformities and handicaps. This may contribute to the high deformity rate observed in large adult wild *C. lumpus*. That some species can survive in nature without a tail fin (*e.g.*, Tyler *et al.*, 2014) probably reflects how much they depend on their caudal fin as a hydrofoil (Nursall, 1958) and their reliance on the dorsal, anal and pectoral fins for locomotion. Moreover, *C. lumpus* are sluggish fish that feed on larger planktonic organisms or benthic invertebrates (Davenport, 1985) and will therefore likely still be able to find food despite compromised swimming capabilities. In addition, owing to their globiform morphology, *C. lumpus* will have a limited number of natural predators once they reach larger sizes, which further helps explain how they can survive in nature with severe deformities.

4.2 | Swelling of specific bones in wild *L. bergylta*

Although the vertebra deformity rate in wild *L. bergylta* was low, 83% of the inspected fish had fractures and calluses in their ribs and/or neural and haemal spines. Recently, Fjelldal *et al.* (2018) described fracture and fracture repair in neural and haemal spines in *G. morhua*, where fractures healed normally by callus formation and remodelling back into a normal structure. Some of the studied *L. bergylta* had abundant callus formation, larger than the calluses reported by Fjelldal *et al.* (2018), but similar to those reported in *M. aeglefinus* (Jawad *et al.*, 2018) and striped piggy *Pomadasys stridens* F. 1775 (Jawad, 2013). Why bone fracture and calluses occur in ribs and neural and haemal spines in wild fish is unclear. However, as these pathologies were site specific this could give an indication as to their cause. For instance, in neural and haemal spines, calluses occurred in the anterior tail region of the vertebral column. This region has high mechanical loading imposed by the swimming musculature and flexion of the vertebral column, which may indicate stress fractures as being the root cause. For example, human rowers are known to develop site-specific stress fractures in ribs due to mechanical stress (McDonnell *et al.*, 2011). In *L. bergylta*, the ribs are not expected to suffer from mechanical loading during swimming, but may suffer from repetitive loading due to food ingestion. We observed abundant calcified masses in the stomachs of radiographed wild *L. bergylta*, presumably due to feeding on shellfish and echinoderms. Subsequently, these hard structures may repetitively load the ribs, inducing stress fracture. Under conditions of repetitive stress some rib fractures may not heal normally, and nonunions of muscular force stress-induced rib fractures occur in human athletes (Proffer *et al.*, 1991). Suh *et al.* (2001) reported a hypertrophic nonunioned rib fracture with abundant callus formation and radiological appearance similar to that later found in haemal spine calluses in wild *M. aeglefinus* (Jawad *et al.*, 2018) and in ribs, and haemal and neural spines of the currently inspected wild *L. bergylta*. Hence, in fish, the repetitive stress caused by body undulation during swimming and ingestion of large hard particles may cause site-specific chronic mechanical stress and stress fractures that develop into nonunions caused by impaired callus formation. Indeed, some wild *L. bergylta* had neural spines with fractures that were clearly displaced. Furthermore, calluses were only observed in wild *L. bergylta* and in the largest size class of cultured *L. bergylta*, which suggests that the risk of fractures and calluses is associated with later stages of development. Nevertheless, the reasons why presumed normal behaviour in a natural habitat may induce stress fractures in some fish species is perplexing and deserves to be studied further.

4.3 | Can stress fracture induce fish hyperostosis?

Resently, both Fjelldal *et al.* (2018) and Jawad *et al.* (2018) discussed a possible link between bone fracture and fish hyperostosis – swollen bone (Korschelt, 1940; Smith-Vaniz *et al.*, 1995). Fish hyperostosis has interested scientists for decades and has been identified in at least 22 families, among them extant or fossilized marine species, and
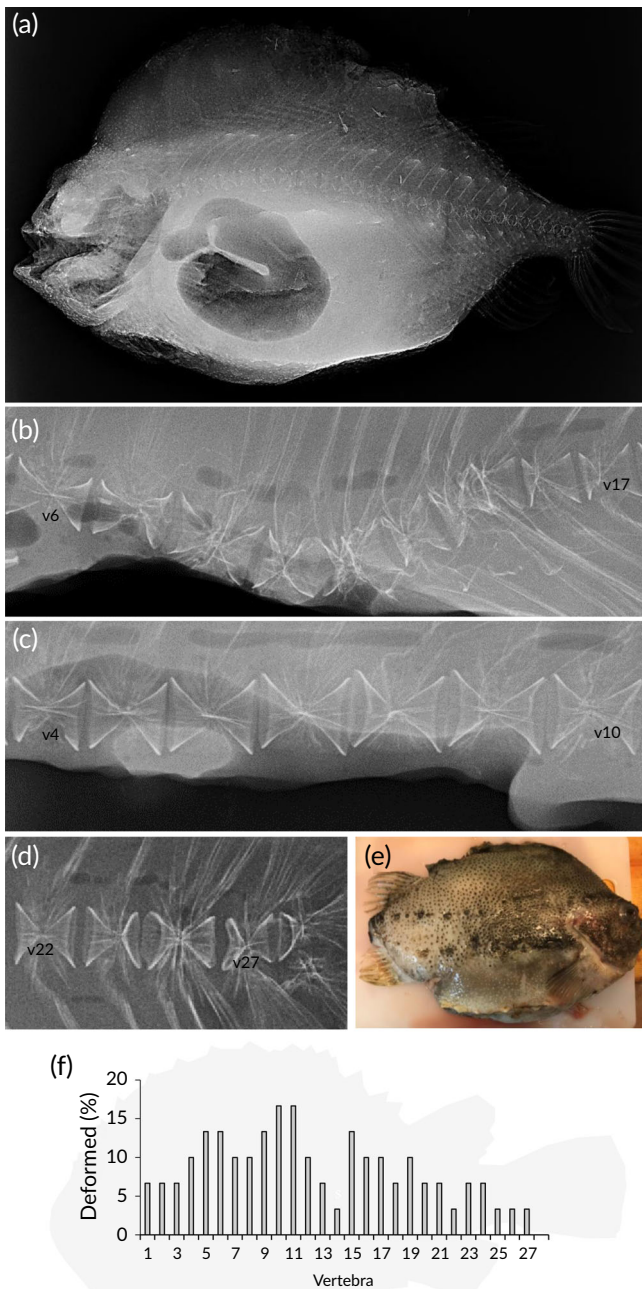
FIGURE 3 Skeletal pathologies in wild lumpfish. (a) Whole skeleton. Note the radiodense structures within the skin. (b) Lordosis was observed in one wild individual. This fish had multiple vertebral compressions and fusions. (c) Vertically shifted vertebrae. (d) Three vertebra that have fused together and remodelled into a single large vertebra. (e) A wild lumpfish of 2.2 kg that was missing the ural part of the vertebral column and the tail fin. The surface of the wound was completely healed. (f) The prevalence of deformed vertebrae along the vertebral column in wild lumpfish

FIGURE 4 Proposed pathogenesis for fish hyperostosis

shows a species-specific site of occurrence (Smith-Vaniz et al., 1995; Smith-Vaniz & Carpenter, 2007). Indeed, the current study shows that the occurrence of haemal and neural spines that are entirely swollen or have fractures and calluses, with and without reduced radiodensity,
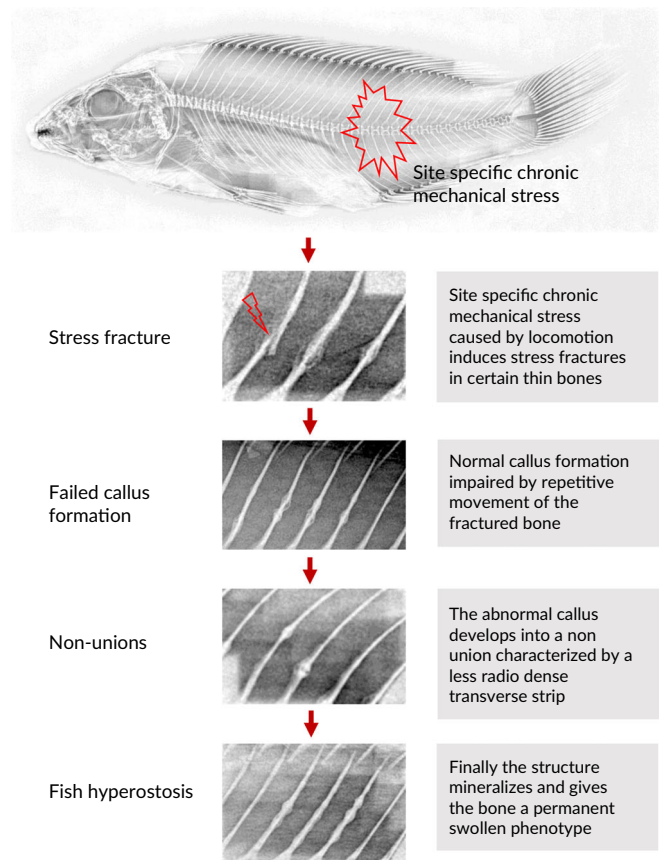
is highly site specific in *L. bergylta*. The current study sets a path for a plausible pathogenesis for site-specific hyperostosis development in fish: site-specific chronic mechanical stress → stress fracture → failed callus formation → nonunions → fish hyperostosis (Figure 4).

4.4 | Site-specific vertebra deformity in cultured *L. bergylta* and low deformity rate in cultured *C. lumpus*

At this stage, it is unclear why cultured *L. bergylta* have a high vertebra deformity rate. In fish vertebrae, the mineral content is important for structural integrity and mechanical strength (Fjelldal et al., 2006; Hamilton et al., 1981). As such, vertebrae with a low mineral content are soft and rubbery (Baeverfjord et al., 1998) and can develop a compressed phenotype (Fjelldal et al., 2007). Although all vertebrae along the vertebral column may have a low mineral content (Fjelldal et al., 2006), mineralization-related deformity development is often site specific (Fjelldal et al., 2009b), and may reflect where the mechanical loading imposed by the lateral musculature is largest and/or the ontological state at which the deformity is induced (reviewed in Fjelldal et al., 2012a). Indeed, the current vertebra deformities in cultured *L. bergylta* were predominately located within two regions, between vertebra 4 and 10 (S1) and between vertebra 19 and 26 (S2). Vertebra fusion dominated S1, while compression dominated S2.

Compressed vertebrae may ultimately fuse, caused by remodelling and mineralization of the ectopic cartilage that occupies the intervertebral space (Witten *et al.*, 2006). Hence, S1 deformities were most probably older than the S2 deformities. Similarly, vertebra deformities in the trunk region of *S. salar* develop earlier in life than those in the caudal region (Grini *et al.*, 2011). Deformity development is a relatively slow process, and there can be a substantial time gap between the deformity induction and development (Fjelldal *et al.*, 2012b; Grini *et al.*, 2011). Hence, the advanced vertebra deformities (S1 fusions) displayed by the smaller cultured *L. bergylta* (~8 g) in the current study show that induction occurred early in development. This is further supported by the present consistent deformity prevalence in cultured *L. bergylta* across all sizes or after periods in sea cages. In conclusion, the results show that the production methods used in *L. bergylta* aquaculture do not support normal vertebra development. As a first step, new studies should focus on early life bone development and mineralization in cultured *L. bergylta* to further understand the causal factor(s) for deformity development.

Considering that *C. lumpus* is a relative new species in aquaculture, the observed low deformity rate is surprising and reflects that the currently used production method supports normal bone development in this species. Looking at the radiographs, the vertebrae of both wild and cultured *C. lumpus* have a low radiodensity, opposite to *L. bergylta* where the radiodensity of the vertebrae is very high. This may reflect that there is a difference in vertebra bone mineral content between the species that may explain why *L. bergylta* seems to be prone to deformity development, while *C. lumpus* are not. However, the mineral content and morphology of vertebrae in wild *L. bergylta* and *C. lumpus* are unexplored. The link between nutrition and skeletal pathology in fish is clear (Baeverfjord *et al.*, 2019; Lall & Lewis-McCrea, 2007; Sugiura *et al.*, 2004), and if the species-specific dietary mineral requirement is not met, vertebrae bone mineralization is lower than normal, leading to deformity development (Fjelldal *et al.*, 2009b).

CONTRIBUTIONS
All authors have read and approved the manuscript. P.G.F designed and completed the study and manuscript, undertook radiology and helped with data analysis. A.M., M.H. and F.O. supplied biological material and helped with manuscript preparation. L.H.S. was project leader, supplied funding and helped with manuscript preparation. T.W.K.F. had the original idea, undertook radiology and data analysis, and helped with manuscript preparation.

ORCID
Per Gunnar Fjelldal https://orcid.org/0000-0001-9237-2706

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