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5
6 **ORIGINAL RESEARCH ARTICLE**

7
8 **Prevalence and Causes of Skeletal Deformities in Cultured**
9 **Juvenile *Oncorhynchus mykiss***

10 *Prevalencia y causas de deformidades esqueléticas en ejemplares juveniles*
11 *cultivados de Oncorhynchus mykiss*

12 *Prevalência e causas de deformidades esqueléticas em exemplares juvenis*
13 *cultivados de Oncorhynchus mykiss*

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24

25 **Abstract**

26 **Background:** Skeletal development and the incidence of skeletal deformities in fish are among
27 the most important problems that need to be solved to increase the success of aquaculture.
28 Skeletal deformities reduce the survival, growth, and nutrition of fish. **Objective:** In this study,
29 skeletal deformations were investigated in juvenile *Oncorhynchus mykiss*, the most widely
30 farmed trout in Turkey. **Methods:** Thirty trout farms were visited, and 1200 juvenile individuals
31 were collected and analyzed. In order to determine the skeletal deformations in the collected
32 samples, the observed deformations were divided into groups. The most common pathological
33 findings in individuals were also evaluated. **Results:** As a result of the study, the most common
34 skeletal deformation was compression (C), with a rate of 49.83%. When analyzed according to
35 region, the most deformation occurred in the tail lordosis-kyphosis region, with a rate of
36 73.17%. On the other hand, when we analyzed the specimens, 61% lordosis was detected. When
37 jawbone deformities, fin deformities, and pterygophore deformities were analyzed, jawbone
38 deformities were found at the highest rate of 12.08 %. The most common pathological finding
39 in the samples was swimming disorder (37.58 %). Another result was the change in total
40 deformation rate depending on different water temperatures in the farms. **Conclusions:**
41 According to the results, the highest deformation rate was found to be 19.58% at 13°. The
42 prevalence of deformities indicates that the environmental conditions in which aquaculture
43 practices are carried out should be regulated as well as the deficiencies in the aquaculture
44 management system.

45 **Keywords:** *juvenile fish; Oncorhynchus mykiss; pisciculture; skeletal deformities.*

46

47 **Resumen**

48 **Antecedentes:** El desarrollo esquelético y la incidencia de deformidades esqueléticas en los
49 peces se encuentran entre los problemas más importantes que deben resolverse para aumentar
50 el éxito de la acuicultura. Las deformidades esqueléticas reducen la supervivencia, el
51 crecimiento y la nutrición de los peces. **Objetivo:** En este estudio, se investigaron las

52 deformaciones esqueléticas en juveniles de *Oncorhynchus mykiss*, la trucha más cultivada en
53 Turquía. **Métodos:** Se visitaron treinta granjas de truchas y se recolectaron y analizaron 1200
54 individuos juveniles. Para determinar las deformaciones esqueléticas en las muestras
55 recolectadas, las deformaciones observadas se dividieron en grupos. También se evaluaron los
56 hallazgos patológicos más comunes en los individuos. **Resultados:** Como resultado del estudio,
57 la deformación esquelética más común fue la compresión (C) con una tasa del 49.83%. Cuando
58 se analizó según las regiones, la mayor deformación se produjo en la región de lordosis-cifosis
59 de la cola con una tasa del 73.17%. Por otro lado, al analizar los especímenes se detectó un 61%
60 de lordosis. Al analizar las deformidades de los huesos maxilares, de las aletas y de los
61 pterigóforos, las deformidades de los huesos maxilares se encontraron con la tasa más alta con
62 un 12.08%. El hallazgo patológico más común en las muestras fue el trastorno de la natación
63 (37.58%). Otro resultado fue el cambio en la tasa total de deformación en función de las
64 diferentes temperaturas del agua en las granjas. **Conclusiones:** De acuerdo a los resultados, la
65 tasa más alta de deformación se encontró con un 19.58% a 13°. La prevalencia de deformidades
66 indica que se deben regular las condiciones ambientales en las que se realizan las prácticas
67 acuícolas, así como las deficiencias en el sistema de gestión acuícola.

68 **Palabras clave:** *deformidades esqueléticas; juvenil; piscicultura; Oncorhynchus mykiss.*

70 **Resumo**

71 **Antecedentes:** O desenvolvimento esquelético e a incidência de deformidades esqueléticas nos
72 peixes estão entre os problemas mais importantes que devem ser resolvidos para aumentar o
73 sucesso da aquacultura. As deformidades esqueléticas reduzem a sobrevivência, o crescimento
74 e a nutrição dos peixes. **Objetivo:** Neste estudo foram investigadas deformações esqueléticas
75 em juvenis de *Oncorhynchus mykiss*, a truta mais cultivada em Türkiye. **Métodos:** Foram
76 visitadas 30 explorações de truticultura e recolhidos e analisados 1.200 indivíduos juvenis. Para
77 determinar as deformações esqueléticas nas amostras recolhidas, as deformações observadas
78 foram divididas em grupos. Os achados patológicos mais comuns nos indivíduos também foram
79 avaliados. **Resultados:** Como resultado do estudo, a deformação esquelética mais comum foi
80 a compressão (C) com uma taxa de 49.83%. Quando analisado por região, a maior deformação
81 ocorreu na região lordose-cifose da cauda com um índice de 73.17%. Por outro lado, ao analisar
82 os espécimes, detetou-se 61% de lordose. Ao analisar as deformidades dos ossos maxilares,
83 barbatanas e pterigóforos, foram encontradas deformidades dos ossos maxilares com uma taxa
84 mais elevada de 12.08%. O achado patológico mais comum nas amostras foi o distúrbio da

85 natação (37.58%). Outro resultado foi a alteração da taxa de deformação total em função das
86 diferentes temperaturas da água nas explorações. **Conclusões:** De acordo com os resultados, a
87 maior taxa de deformação foi encontrada com 19.58% no 13°. da aquicultura.

88 **Palavras-chave:** *deformidades esqueléticas; juvenil; Oncorhynchus mykiss; piscicultura.*

89

90 **Introduction**

91 The aquaculture sector is experiencing rapid growth on a global scale with a significant impact
92 on food security (Kebede and Habtamu 2016). Aquaculture production demonstrated a 6.2%
93 increase in 2022 compared to the previous year, reaching 849,808 tons. Of the total production,
94 30% was constituted by marine fish obtained through hunting, 5.6% by other marine products
95 obtained through hunting, 3.9% by inland aquatic products obtained through hunting, and
96 60.6% by aquaculture products (Tuik, 2023). Deformities in fish are a common and serious
97 problem in aquaculture and pose an economic challenge (Noble *et al.*, 2012). The study of these
98 abnormalities and deformations has a long history, dating back to the sixteenth century. Since
99 that time, a large number of studies have been conducted, reporting a variety of deformities
100 observed in fish (Jawad *et al.*, 2016). Deformities have a significant impact not only on
101 aquaculture operations but also on the global conservation and survival of endangered fish
102 species (Chandra *et al.*, 2021). The most important deformities are the spinal deformities, which
103 are vital structural components that affect swimming. Indeed, spinal deformities have the
104 potential to highly affect swimming performance (Powell *et al.*, 2009). Spinal deformities can
105 occur with different types of vertebral body deformities and different types of curvatures that
106 occur together or separately (Witten *et al.*, 2009). It has been determined that skeletal
107 malformations affecting the head, spine, and appendicular skeleton are related to the rearing
108 style and conditions. As a result, the economic importance of such deformities is clear.
109 However, jaw abnormalities such as upper or lower jaw torsion and upper or lower jaw
110 extension impair the feeding ability of the fish. This can eventually lead to starvation and fish
111 death (Boglione *et al.*, 2013). Various factors, such as physico-chemical properties of the water
112 (temperature, pH, salinity, water flow rate), pollution, nutrition, genetic factors, and aquaculture
113 infrastructure, cause deformities (Fopp *et al.*, 2022). Identification of deformities can provide
114 fish farms with a tool to predict abnormalities in adult stages or allow modification of abiotic
115 or biotic factors to improve sample quality during growth (Yıldırım *et al.*, 2014). In this study,
116 deformities occurring at the juvenile stage in rainbow trout farms were analyzed, and this study
117 aims to identify their causes.

118 **Materials and methods**

119 *Ethical statement*

120 The authors declare that all ethical rules were followed throughout the research. The study does
121 not have any requirement for ethical approval.

122 *Fish Sampling*

123 Thirty trout farms were visited during the study. Deformed individuals with an average live
124 weight of 5-15 g were sampled. A total of 1200 samples were analyzed. Water temperatures in
125 the farms varied between 9 C°-13 C°. The oxygen concentration of the water samples was found
126 to be in the range of 8.9 to 8.5 mg/L (Table 1).

127

128 **Table 1.** The number of farms included in the study, the parameters used to assess water quality,
129 and the number of samples collected from each farm are presented in the table.

	Temperature of the aquaculture water	Level of oxygen in the water	Number of farms sampled	Number of fish collected from farms	Total number of samples	Average live weight of fish
A Farms	9°	8.9 mg/L	8	40 fish	320 fish	5-15 gr
B Farms	10°	8.9 mg/L	8	40 fish	320 fish	5-15 gr
C Farms	11°	8.7 mg/L	4	40 fish	160 fish	5-15 gr
D Farms	12°	8.7 mg/L	5	40 fish	200 fish	5-15 gr
E Farms	13°	8.5 mg/L	5	40 fish	200 fish	5-15 gr

130

131 The table below presents the feeds and nutritional contents used in fish farming. The feeds
132 utilized in fish farming operations are sourced from a range of commercial entities, and their
133 nutritional profiles exhibit considerable variation (Table 2).

134

135 **Table 2.** The feeds and nutritional contents utilized in fish farming operations.

136

	Feed 1 (A farms)	Feed 2 (B Farms)	Feed 3 (C farms)	Feed 4 (D and E farms)
Protein Content	57.3 %	55.4 %	55.4 %	55.4 %
Crude fat	12.5 %	14.89 %	12.5 %	11.5 %
Cellulose	0.5 %	1.59 %	1.2 %	0.6 %
Crude Ash	13 %	12.19 %	12 %	11 %
Phosphorus	1.99 %	1.80 %	1.60%	1.5 %

Calcium	3.20	3.21%	3.20%	3.00 %
Lysin	3.30 %	3.25 %	3.30 %	3.75 %
Metionin	1.80 %	1.55 %	1.80 %	2 %
Vitamine A	4500 ui/kg	5200 ui/kg	5250 ui/kg	5250 ui/kg
Vitamine D3	1125 ui/kg	1215 ui/kg	1230 ui/kg	1235 ui/kg
Vitamin E	225 mg/kg	215 mg/kg	220 mg/kg	225 mg/kg
Vitamin C	165 mg/kg	155 mg/kg	165 mg/kg	165 mg/kg

137

138

139 *Clinical examination*

140 The most notable pathological findings observed during the sampling process at fish farms were
 141 swimming disorders, general weakness, and exophthalmus. A clinical examination was
 142 conducted on the samples, and the results were recorded. In order to determine the skeletal
 143 deformations in the samples collected, the deformations observed were divided into groups.
 144 The first group; Compression (C), Fusion (F), Compression and Fusion (C&F), Fusion center
 145 (FC), dorsally wedged vertebrae (W), compressed and dorsally wedged vertebrae (C&W)
 146 Reduced intervertebral space (R) (Mariasingarayan *et al.* 2024). The second group is defined
 147 as jaw bone malformation (JBM), fins (F), pterygophore malformation, epural malformation,
 148 and hypural malformation. X-rayed with an EcoRay Toshiba® apparatus. The presence of
 149 skeletal deformations was established through a detailed examination of the samples.

150

151 **Results**

152 1200 samples collected from farms were analyzed. As a result of the examination, Compression
 153 (C) was found to be the most common skeletal deformation, with a rate of 49.83%. This was
 154 followed by fusion center (48.33%; FC), compression and fusion (30.83%; C&F), fusion
 155 (29.75%; F), compressed and dorsally wedged vertebrae (22.75%; C&W), reduced
 156 intervertebral space (16.58%; R), dorsally wedged vertebrae (5.75%; W). When analyzed by
 157 region, the highest deformation occurred in the lordosis-kyphosis region of the tail, with a rate
 158 of 73.17%. The deformation rates in the whole-body kypho-lordo-kyphosis and tail region
 159 kypho-lordosis regions were 66.67% and 64%, respectively (Figure1, Table 3).

160

161 **Table 3.** Vertebral (V) deformity classification of *Oncorhynchus mykiss* species collected
 162 from farms.

	Vertebral column category	Compression (C)	Fusion (F)	Compression and Fusion (C&F)	Fusion center (FC)	Dorsally wedged vertebrae (W)	Compressed and dorsally wedged vertebrae (C&W)	Reduced intervertebral space (R)
Tail region lordo-kyphosis (Total :73.17%)	Platyspondyly	112	55	33	4	7	51	26
	Normal	58	32	21	19	3	23	12
	Lordosis	79	21	17	44	5	33	15
	Kyphosis	43	35	45	65	1	12	7
	Total	329	162	143	164	25	138	79
	%	24.33	11.92	9.67	11.00	1.33	9.92	5.00
Tail region kypho-lordosis (Total :64%)	Platyspondyly	52	44	22	22	2	27	11
	Normal	23	27	54	67	12	16	9
	Lordosis	66	34	51	21	7	25	21
	Kyphosis	31	19	47	11	7	17	23
	Total	213	137	213	148	40	106	95
	%	14.33	10.33	14.5	10.08	2.33	7.08	5.33
Whole body kypho-lordo-kyphosis (Total :66.67%)	Platyspondyly	51	24	38	76	6	21	15
	Normal	38	11	9	32	5	19	32
	Lordosis	27	32	13	178	11	21	11
	Kyphosis	18	23	20	41	3	8	17
	Total	149	107	97	386	43	86	86
	%	11.17	7.50	6.667	27.25	2.08	5.75	6.25
Total	%	49.83	29.75	30.83	48.33	5.75	22.75	16.58

163



164 **Figure 1.** Lordosis, kyphosis, and scoliosis (a, b, c), deformation of the upper jaw (d),
 165 deformation of the lower jaw (e and f), and spinal curvatures (g, h) are observed.

166

167 On the other hand, when we examined the specimens, lordosis was found in 61%. This was
 168 followed by scoliosis (41.67%) and kyphosis (41.08%) (Figure 1, 2). In jaw bone deformities,
 169 fin deformities, and pterygophore deformities, jawbone deformities were found in 12.08%. This
 170 was followed by Fins deformities with 8.25% and Malformed pterygophore with 4.42%. In
 171 epural and hypural deformities, epural deformities were noted in 44.33% (Figure1, 2).

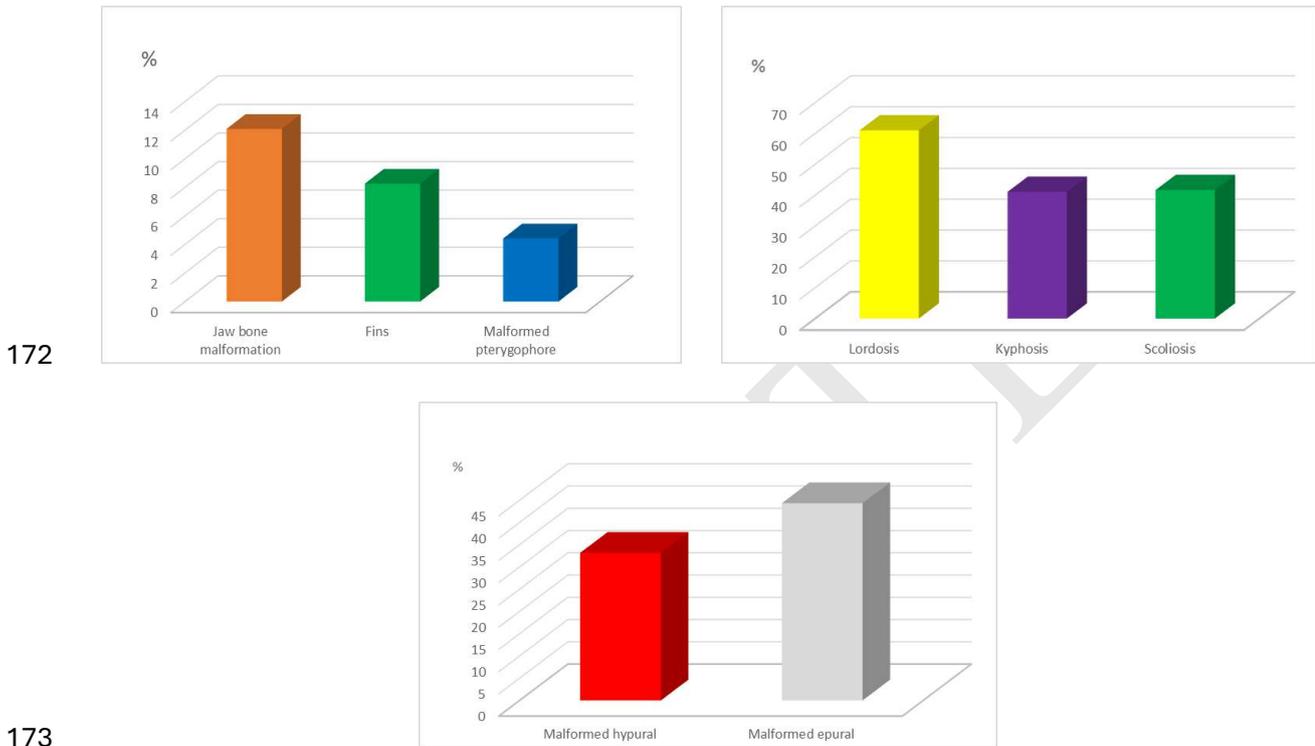
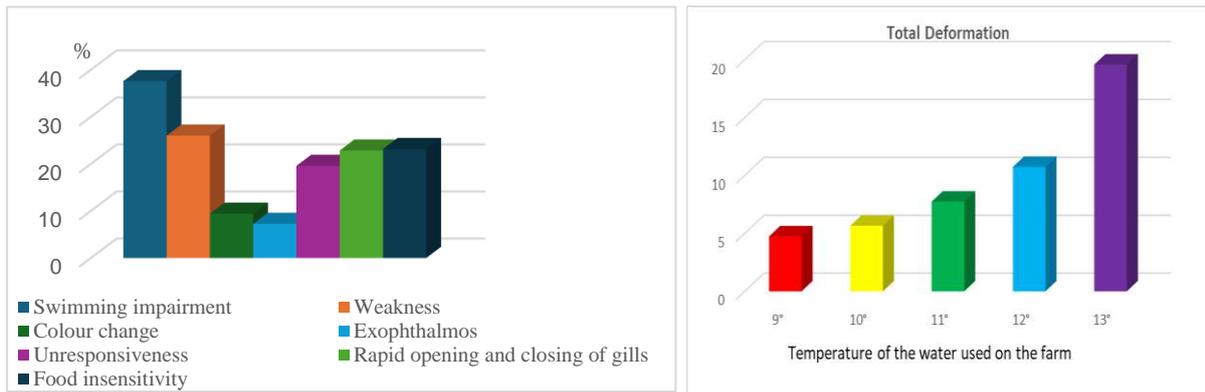


Figure 2. Rates of other malformations detected.

176 The most common pathological finding in the samples collected was impaired swimming
 177 (37.58%). This was followed by weakness (26%), food insensitivity (23.27%), rapid opening
 178 and closing of gills (22.83%), unresponsiveness (19.58%), color change (9.42%), and
 179 exophthalmos (7.25%) (Figure 3). A further outcome of note is the alteration in the overall
 180 deformation rate in response to disparate water temperatures within the farms environment.
 181 Pursuant to the findings, it was determined that the maximum deformation registered at 19.58%
 182 at an angle of 13C°. This was followed by 12C° (10.75%), 11C° (7.75%), 10C° (5.67%) and
 183 9C° (4.75%) (Figure 3).



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Figure 3. The most common findings in the samples and Total deformation by water temperature in farms

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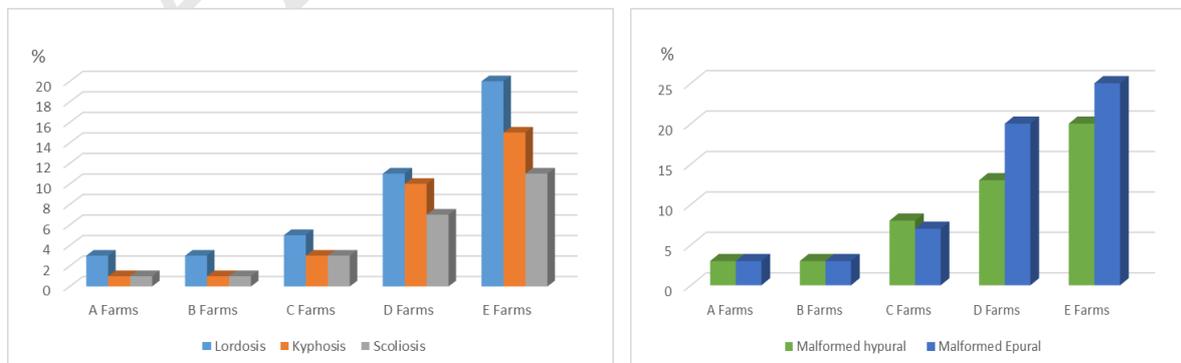
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As indicated in the table presented in Material Method, the farms were classified into five distinct groups. The data in this table demonstrate that the water temperatures, oxygen levels, and commercial feeds utilized by the farms exhibit notable variation. Consequently, the farms were evaluated as individual units within their respective groups, and the observed skeletal deformations are presented in this section. The highest rates of lordosis, kyphosis, and scoliosis were observed among fish farm groups, with 20%, 15%, and 11% of farms in group E exhibiting these deformations, respectively. In comparison, the lowest percentages of these deformations were observed in farms in group A. (Figure 4) The most significant deformations, characterized by jawbone malformation and fins, as well as malformed pterygophore, were identified with the highest incidence and at the same rate in fish farms within groups D and E. Conversely, the lowest prevalence of these deformations was observed in fish farms within group A (Figure 4) The prevalence of other noteworthy deformities, namely malformed hypural and epural, was found to be the lowest in fish farms belonging to groups A and B, while the highest rates were observed in those falling under groups D and E (Figure 4.)

201



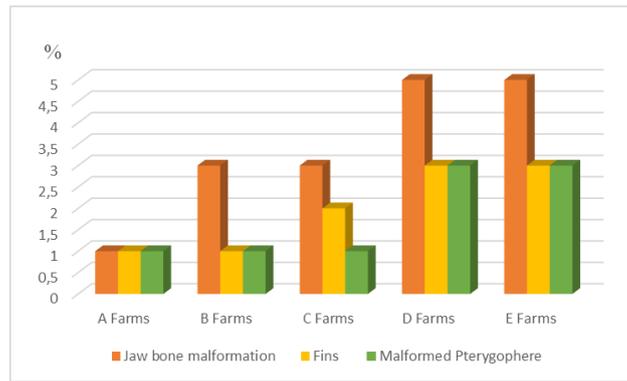


Figure 4. Fish farm deformation rates

Discussion

The occurrence of skeletal deformities during fish growth and development does not only affect the external morphology. However, impaired mobility can result in growth retardation, malnutrition, and ultimately death. Fish with deformed morphology are often discarded during harvest because they are not desirable for consumers. Such events lead to significant economic losses in farms. These losses have been documented by Berillis *et al.* (2015) and Fragkoulis *et al.* (2019). Many types of deformations are observed in fish. The most common deformations are kyphosis, scoliosis, lordosis, mandibular deformities, and vertebral fusion (Eissa *et al.*, 2009). A plethora of factors have been posited as potential etiological agents for these malformations, including, but not limited to, nutritional deficiency, environmental conditions, the presence of toxic substances, physical restraint, agricultural practices, disease, and genetic factors (Ashaf *et al.*, 2021). The investigation revealed that Compression (C) was the most prevalent skeletal deformity, with a prevalence rate of 49.83%. Subsequently, the most prevalent deformities were fusion center (48.33%; FC), compression and fusion (30.83%; C&F), fusion (29.75%; F), compressed and dorsally wedged vertebrae (22.75%; C&W), and reduced intervertebral space (16.58%; R). The least common deformities were dorsally wedged vertebrae (5.75%; W). Upon analysis by region, the highest deformation was observed in the lordosis-kyphosis region of the tail, with a rate of 73.17%. The deformation rates in the kypho-lordo-kyphosis regions of the whole body and the kypho-lordosis region of the tail were 66.67% and 64%, respectively (Table 1). Many studies have reported the occurrence of skeletal deformities in fish species (Lv *et al.*, 2019). One of the most important challenges in intensive aquaculture is the abundance and prevalence of skeletal malformations, especially lordosis,

227 kyphosis, scoliosis, and vertebral fusion. The aetiology of these deformities is multifactorial.
228 The most important of these factors are nutrition, environmental conditions, farming practices,
229 and genetic factors (Georgakopoulou *et al.*, 2010). The most common and most common
230 external deformities in fish are kyphosis, scoliosis and lordosis, which can be seen in the
231 abdominal region of the vertebral column. These deformities can be detected at an early stage.
232 As a result, these deformities can be evaluated externally during rearing, and if necessary, fish
233 showing such deformities can be removed from the population before they reach marketable
234 size. Other malformations may be observed, including deformities of the pterygiophores of the
235 dorsal and anal fins, vertebral fusion, and deformities of the caudal complex. These
236 malformations do not affect the external shape of the fish but can be observed during rearing
237 (Boglione *et al.*, 2013). Indeed, the study revealed that lordosis was present in 61% of the
238 subjects. This was followed by scoliosis (41.67%) and kyphosis (41.08%) (Figure 2). In the
239 case of jawbone deformities, fin deformities, and pterygophore deformities, it was found that
240 12.08% of the subjects exhibited jawbone deformities. The next most prevalent deformities
241 were fins, with an incidence of 8.25%, and malformed pterygophores, with an incidence of
242 4.42%. The development of the head and jaw is a complex and difficult process that involves
243 the coordinated operation of multiple genes (Delaurier *et al.*, 2019). A study conducted by
244 Babaheydari *et al.* (2016) analyzed hatched rainbow trout (*Oncorhynchus mykiss*) larvae to
245 determine differences in protein expression between normal and deformed fish. This study
246 provided preliminary information on the molecular mechanisms underlying skeletal
247 malformations in fish by identifying nine protein regions that were altered in deformed fish.
248 However, the authors also emphasized that further validation studies using mRNA expression
249 techniques or antibody-based analyses are needed to use these proteins as indicators of skeletal
250 deformity. Such studies are of great importance in understanding the relationship between
251 skeletal deformity and genetic factors. A review of the available evidence revealed that epural
252 and hypural deformities were present in 44.33% (Figure 2) of the specimens examined. The
253 structural integrity and durability of the mineral content play an important role in the protection
254 of fish spines (Fjelldal *et al.*, 2018). As a result, vertebrae with low mineral content are hard
255 and weak, which may lead to the development of a compressed formation. Furthermore, despite
256 the fact that all vertebrae along the vertebral column exhibit low mineral content, the
257 development of mineralization-related deformities is, as a general rule, regional. This
258 phenomenon may be indicative of the location where the load exerted by the lateral muscles is
259 greatest, and the ontological situation in which the deformity occurs. (Fjelldal *et al.*, 2021). As

260 previously indicated, specific deformities have been identified in cultured fish species, which
261 have the potential to impact various biological processes, including growth, swimming, feeding,
262 resistance, speed, and survival (Berillis 2017). It can be reasonably inferred that the
263 pathological findings observed in the fish in this study provide support for this hypothesis. The
264 most frequently observed pathological condition in the sampled fish was impaired swimming,
265 which occurred in 37.58% of cases. The most prevalent pathological manifestations were
266 observed to be weakness (26%), food insensitivity (23.27%), the rapid opening and closing of
267 gills (22.83%), unresponsiveness (19.58%), color change (9.42%), and exophthalmos (7.25%)
268 (Figure 3). Severe skeletal deformities mean the selective removal of individuals with
269 significant deformities from the population. Such practices cause significant economic losses
270 for the stabilization and preservation of stock quality (Rutkayov *et al.*, 2016). Studies have
271 determined the existence of biotic and abiotic factors underlying skeletal deformities in fish
272 (Park *et al.*, 2016). Changes in water temperature can affect skeletal formation and lead to
273 morphological malformations (Fraser *et al.*, 2015). Another important area of interest in
274 biological research is the effect of environmental factors on the developmental processes of
275 fish. Temperature is the most important factor affecting fish development, including survival,
276 nutrition, and possible deformities (Fraser *et al.*, 2015). Accordingly, temperature has been
277 determined as the "main abiotic factor" for the development of fish (Güralp *et al.*, 2017). In
278 addition, temperature level has an important effect on body deformities, nutrition, ontogenetic
279 development, and growth and survival of fish (Kim *et al.*, 2018). An increase in water
280 temperature has been reported to be an effective method to increase growth rate and production
281 in cold water aquaculture. However, this method has also shown that high temperatures are a
282 factor contributing to the increased frequency of skeletal deformities (Ytteborg *et al.*, 2010). A
283 review of the scientific literature reveals that the optimal water temperature for *O. mykiss*, a
284 type of rainbow trout, is 10°C. Temperatures above 14 °C are detrimental, and below 6 °C are
285 suboptimal. The most common skeletal malformations observed in rainbow trout
286 (*Oncorhynchus mykiss*) are compression or fusion vertebrae (Lein *et al.*, 2009). *Pagellus*
287 *erythrinus* displays a 75% prevalence of deformities in unfavorable temperature conditions due
288 to its developmental plasticity (Sfakianakis *et al.*, 2004). The present study revealed that an
289 elevated rate of deformity was observed in organisms as the cultivation temperatures increased.
290 The change in total deformation rate due to different water temperatures in the farms was found
291 to be 19.58% at 13°, followed by 12° (10.75%), 11° (7.75%), 10° (5.67%) and 9° (4.75%)
292 (Figure 3). This result is corroborated by the findings of Han *et al.* (2020), which revealed a

293 greater prevalence of deformities in *T. ovatus* at 32°C. Among the observed deformities, the
294 most prevalent was lordosis of the vertebral column in the caudal region of the organisms,
295 occurring in 61% of cases. This is in accordance with the findings of Andrades et al. (1996),
296 who identified lordosis as the most prevalent type of deformity among fish. A balanced diet,
297 including an appropriate quantity and proportion of nutrients, such as protein, carbohydrates,
298 lipids, vitamins (A, D, E, K, and C), and minerals (phosphorus, calcium, and trace elements),
299 is essential for the optimal growth and development of fish. Any deviation from the optimal
300 level, excess or deficiency of nutrients, can impair growth and development and lead to
301 deformities in fish. Malnutrition has been identified as a significant contributing factor to
302 deformities in fish. It has been demonstrated that inadequate nutrition can result in
303 malformations, particularly at an early developmental stage (Liang *et al.*, 2017). A deficiency
304 of vitamins C and E, for instance, can lead to bone and muscle disorders in fish, a phenomenon
305 that has been demonstrated by Näslund et al. (2016) and other research studies. Deficiencies in
306 vitamins C and K have similarly been associated with skeletal deformities in fish, with evidence
307 suggesting that they affect bone mineralization in several species (Darias *et al.*, 2011).
308 Deficiencies in vitamin C in diets have been linked with the occurrence of spinal deformities,
309 including lordosis, in a range of species, including rainbow trout (*Oncorhynchus mykiss*),
310 Japanese halibut (*Paralichthys olivaceus*) and cichlids (Berillis *et al.*, 2015). Furthermore,
311 deficiencies in vitamin C have been associated with the development of scoliosis and lordosis
312 in channel catfish (*Ictalurus punctatus*). Conversely, a variety of biotic factors, encompassing
313 pathogenic infections, genetic predisposition, and the circumstances under which fish are bred
314 or reared in captivity, can give rise to deformities in fish. It has been demonstrated that some
315 factors result in the formation of malformations in both adult fish and their offspring, while
316 others have been found to only affect the latter. Disease outbreaks and infections have been
317 observed to cause a range of malformations in fish, including kyphosis, cranial cavitation, and
318 spinal and skeletal anomalies. (Chandra *et al.*, 2024)

319 Deformations of various types are commonly observed in fish specimens. Among the
320 most frequently occurring deformations are kyphosis, scoliosis, lordosis, mandibular
321 deformities, and vertebral fusion (Eissa *et al.*, 2009). The etiology of these malformations has
322 been associated with a multitude of potential causes, including nutrient deficiency,
323 environmental conditions, toxic substances, physical restraint, farming practices, disease, and
324 genetic factors (Ashaf *et al.*, 2021). A plethora of studies have demonstrated a correlation
325 between fish deformities and the presence of heavy metals. Such deformities have been shown

326 to have a profoundly deleterious effect on fish populations, impacting their survival, growth
327 rate, welfare, and external morphology. Fish deformities, particularly those affecting the
328 skeletal system, are of particular concern as they can hinder an organism's capacity to interact
329 effectively with its environment (Demir *et al.*, 2024).

330 Indeed, the study revealed notable discrepancies in the prevalence of severe skeletal
331 deformations across different fish farms. A review of the results indicates that the observed
332 differences may be attributed to the aforementioned factors. A comparative analysis of
333 aquaculture farms indicates that the most significant variations are observed in the composition
334 of their feed and the temperature regimes under which the fish are cultivated. The study revealed
335 that skeletal deformations were the least prevalent on farms where the water temperature was
336 maintained at 9°C. In 2009, Lein provided support for our study by stating that 10°C was the
337 optimal temperature for rainbow trout, 14°C was too high, and that fused or compressed
338 vertebrae were the most common skeletal malformations observed in farms conducted at high
339 temperatures. The findings of this study indicate that an increase in water temperature is
340 associated with a rise in the prevalence of skeletal deformities. The water temperature exerts a
341 considerable impact on the incidence of jaw deformities in the golden pompano (*Trachinotus*
342 *ovatus*). The prevalence of these deformities is observed to be at its peak at a temperature of
343 33°C and at its lowest point at 26°C, indicating a clear correlation between jaw deformity and
344 high water temperature (Lein, 2009). The highest incidence of jaw malformation was observed
345 in Group E farms with the highest water temperature, while the lowest incidence was observed
346 in Group A farms with a water temperature of 9°C. A further significant factor to be considered
347 in the study is the diverse range of feeds provided to the fish. The nutritional composition of
348 each feed differs. It is essential to provide fish with an appropriate quantity and ratio of different
349 nutrients, including protein, carbohydrates, lipids, vitamins (A, D, E, and C), and minerals
350 (phosphorus, calcium, and trace elements), to ensure normal growth and development (Cahu *et*
351 *al.*, 2003) Any deviation from the optimal level, whether excess or deficiency of nutrients, may
352 impair growth and development, and induce deformities in fish. Malnutrition represents a
353 significant contributing factor to the development of deformities in fish. Insufficient nutrition,
354 particularly during the early stages of development, has been identified as a key factor in the
355 emergence of malformations (Liang *et al.*, 2017). A deficiency of essential nutrients, such as
356 vitamins C and E, can lead to bone and muscle disorders in fish (Näslund *et al.*, 2016). A
357 deficiency in vitamin C has been observed to result in the development of lordosis in several
358 species of fish, including rainbow trout (*Oncorhynchus mykiss*), Japanese flounder

359 (*Paralichthys olivaceus*) and cichlids (Darias *et. al.*, 2011). Vitamin A deficiency increased the
360 incidence of mouth deformities in the Japanese flounder, *P. olivaceus*. Hernandez And Hardy
361 (2020) discovered that elevated levels of vitamin A in dietary regimens may disrupt the typical
362 coordination between bone development and bone matrix formation, leading to the emergence
363 of skeletal deformities. Vitamin A has a beneficial effect on the maturation of chondrocytes and
364 the activity of osteoclasts. It also has an enhancing impact on vertebral column development
365 and a suppressive effect on bone matrix formation, which can result in vertebral and jaw
366 deformities. The primary components of bones are inorganic salts, calcium, and phosphorus.
367 Deficiency of phosphorus results in a range of deformities, including growth retardation,
368 skeletal deformities, soft bones and a curved spine in Atlantic salmon (*Salmo salar*), cephalic
369 anomalies of the frontal bones in mirror carp, and twisted neural and haemal spines in Atlantic
370 halibut and juveniles of haddock. The study revealed notable differences in the levels of various
371 vitamins, including Vitamin A, Vitamin D3, Vitamin E, and Vitamin C, in the commercial feeds
372 provided to the fish farm groups. The highest rates of skeletal deformation are observed in the
373 D and E fish farm groups, which were fed the greatest quantity of vitamin A in the commercial
374 feed. The elevated water temperature in these farms, along with the findings of Hernandez and
375 Hardy (2020), which indicated that elevated vitamin A levels in dietary regimes can disrupt the
376 typical coordination between bone development and bone matrix formation, thereby
377 accelerating skeletal deformities, are both confirmed. Conversely, an examination of the
378 phosphorus and calcium rates in the feeds reveals that these are low in the same groups. A
379 deficiency of phosphorus can result in growth retardation, skeletal deformities, soft bones, and
380 a curved spine in Atlantic salmon (*Salmo salar*) (Silverstone, 2002). The combination of a low
381 level of these components, a high water temperature, and a high value of vitamin A may have
382 contributed to the increased prevalence of skeletal deformities observed in fish fed with these
383 feeds. It can be reasonably deduced that the aforementioned factors may have contributed to
384 the prevalence of deformities observed in the fish farms in question. The impact of fatty acids,
385 particularly DHA, on developmental processes in mammals and amphibians is becoming
386 increasingly clear. Dietary fat exerts a profound influence on gene expression, leading to
387 alterations in metabolic processes, growth patterns, and cell differentiation. Fatty acids, and
388 particularly highly unsaturated fatty acids, act on the genome through specific nuclear receptors
389 such as Peroxisome Proliferator Activated Receptors (PPARs). This receptor binds to DNA as
390 a heterodimer with the retinoid X receptor (RXR), which regulates genes involved in skeletal
391 development during ontogeny. It may therefore be hypothesized that highly unsaturated fatty

392 acids affect skeletal formation during development in this way (Cahu *et. al.*, 2003). Upon
393 examination of the feed groups in light of the aforementioned information, it becomes evident
394 that the lowest crude fat content is observed in the feeds provided to fish in groups D and E.
395 Consequently, it can be postulated that deformation may have been a prevalent phenomenon in
396 fish fed with this particular feed.

397

398 **Conclusion**

399 The study revealed a correlation between elevated water temperatures and increased skeletal
400 deformation. The lowest levels of deformation were observed in fish farms with a water
401 temperature of 9°C in group A. The deformation observed was found to be affected by both
402 water temperature and the nutrient content of commercial feeds. Further studies are required to
403 investigate the levels of vitamin A and vitamin D3 present in commercial feeds. It can be posited
404 that an excess of Vitamin A is a contributing factor to skeletal deformation in the presence of
405 elevated water temperatures. It is also important to consider the levels of phosphorus and
406 calcium, which have been linked to deformations. The necessity for the rapid development of
407 cost-effective, farmer-friendly, and robust methodologies for the early detection of deformities
408 in fish and the determination of their causative factors cannot be overstated. The prevalence of
409 deformities indicates the presence of shortcomings in the system of aquaculture management,
410 as well as the environmental conditions under which aquaculture practices are conducted.

411

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413

414 *Funding*

415 The authors declare that they have not received financial resources for the conduct of this
416 research.

417

418 *Data availability*

419 All data generated or analyzed during this study are included in this article.

420

421 *Conflict of Interest*

422 The author declares that there is no conflict of interest related to this work.

423

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