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Ray-Resorption Syndrome in European Seabass, *Dicentrarchus labrax* (Linnaeus, 1758)

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ABSTRACT

Fin abnormalities are common in reared fish. They mainly consist of partial to complete lack of rays and severe abnormalities of fin-supporting skeletal elements, which develop during the larval stage, up to the completion of fin skeleton ontogeny. This study reports a new abnormal condition, ray-resorption syndrome (RSS), which developed after the completion of fin ontogeny in initially normal European seabass larvae, leading to extensive loss of fin rays. At 49 days post-fertilisation (dpf) (15 mm standard length, SL) all fish presented normal fins. However, nineteen days later (21 mm SL), significant fin damage, characterised by ray loss or fractures, was observed in all studied populations. The dorsal fin was most affected (55%–84%), followed by the pelvic (27%–53%) and anal fins (7%–17%). Microscopically, multiple non-mineralised areas resembling resorption lacunae were evident along all fin lepidotrichia. By 98 dpf (40 mm SL), the fin-ray loss had advanced, reaching its higher frequency in the dorsal (74%–83%) and caudal fins (71%–94%). Gene expression analysis revealed a significant upregulation of *bglap* (osteoblast maturation marker), *acp5a* (osteoclast maturation marker) and *mmp13a* (extracellular-matrix remodelling marker) in RSS specimens. The results are discussed in respect of the possible causative factors of RSS.

1 | Introduction

The development of skeletal abnormalities in reared fish is a significant cause of inferior product quality and animal welfare, as well as of lowered hatchery productivity. The mean frequency of abnormalities in commercial hatcheries may reach 20%, but occasionally (e.g., in single production batches or cycles) this incidence may elevate to even 45%–100% (Koumoundouros 2010). Most abnormality types develop during the critical period from the embryo stage to the end of metamorphosis and the completion of skeletal ontogeny (Koumoundouros 2010), due to abiotic (e.g., Cobcroft and Battaglene 2009; Sawada et al. 2018; Kourkouta et al. 2021; Printzi et al. 2021) nutritional (e.g., Cahu et al. 2009; Fernández et al. 2018; Bæverfjord et al. 2019; Kasprzak, Ostaszewska, and Wagner 2019; Sivagurunathan et al. 2022) and genetic factors (e.g., Bardon et al. 2009; Fragkoulis et al. 2020; Aydin et al. 2022). In commercial hatcheries, skeletal abnormalities present a highly variable typology and frequency, often indicative of inadequate control of variations in critical rearing parameters (Boglione et al. 2013; Cavrois-Rogacki et al. 2021; Kourkouta et al. 2022).

Fin abnormalities are frequently reported in reared fish, mainly in the form of missing rays and severe abnormalities of their supporting elements (incomplete fin development) (Georga et al. 2011; Kourkouta et al. 2021; Yue et al. 2022). The partial

to complete lack of the dorsal fin (saddleback syndrome, SBS) is the most studied fin abnormality in reared fish. In different species, it has been recorded to develop in a close association with incomplete development of the paired (*Sarotherodon aureus*, Tave, Bartels, and Smitherman 1983), caudal (*Dentex dentex*, Koumoundouros, Divanach, and Kentouri 2001), anal and pelvic fins (*Dicentrarchus labrax*, Fragkoulis et al. 2017). In reared fish, SBS develops during the larval stage, up to the completion of fin skeleton ontogeny (Koumoundouros, Divanach, and Kentouri 2001; Fragkoulis et al. 2017; Yue et al. 2022). To our knowledge, existing literature on the causative factors of incomplete fin development in reared fish is limited to the effects of dietary retinoids (e.g., caudal fin in *Paralichthys olivaceus*, Haga, Takeuchi, and Seikai 2002; SBS and pelvic fins in *D. labrax*, Mazurais et al. 2009), water temperature (caudal fin in *Sparus aurata*, Kourkouta et al. 2021), intensity of larval rearing methodology (SBS and caudal fin in *D. dentex*, Koumoundouros, Divanach, and Kentouri 2001) and genetic background (SBS and paired fins in *S. aureus*, Tave, Bartels, and Smitherman 1983). Incomplete fin development should not be confused with the damage of normally developed fins (fin damage or fin erosion), which takes place during the rearing period following metamorphosis (i.e., after the completion of fin ontogeny) (Hoyle et al. 2007; Ellis et al. 2008; Weirup, Schulz, and Seibel 2022). Fin damage is attributed to a variety of factors, physicochemical (e.g., nipping, handing, transport, water quality), nutritional or biotic (e.g., stress, infections) (reviewed by Ellis et al. 2008).

Kourkouta et al. (2022) recently described a new abnormal condition (ray-resorption syndrome, RSS) affecting the fin rays of *S. aurata* metamorphosing larvae. The abnormality was present in 7 out of 74 larval examined populations, with a very high frequency (> 90%), in the form of multiple non-mineralised areas scattered along the fin rays. RSS was not linked to any abnormalities of the fin-supporting elements, or to alterations of the non-mineralised matrix of the lepidotrichia. No evidence exists on whether RSS may result to long-lasting changes in fin morphology, or whether the abnormal mineralisation pattern of fin rays can recover in the following ontogenetic period. In the present study we (a) report the presence of RSS in three *D. labrax* populations, which were developed under different temperature conditions, (b) study the evolution of RSS up to the early juvenile period and (c) examine whether RSS is marked with differences in the expression of genes involved in bone formation and resorption.

2 | Materials and Methods

2.1 | Experimental Fish

Six groups of fish were subjected in duplicate to 14°C, 17°C or 20°C water temperature, from the epiboly stage to the end of yolk-sac larval stage (8–11 days post-fertilisation, dpf). All groups were then reared under common conditions and 17°C water temperature (Table S1). At 55 dpf (ca 17 mm standard length, SL) the replicates of each treatment were pooled into one common population and transferred to three 10 m^3 tanks, at 19°C water temperature (Table S2), for pre-growing up to the age of 98 dpf (ca 40 mm SL). Fish were acclimated to the test temperatures at a rate of 0.5°C h^{-1} and to the common temperature of larval rearing at a rate of approximately 0.2°C h^{-1}. Temperature adjustment to the test levels was performed by means of chillers and heaters.

Eggs came from a single spontaneous spawn of captive breeders. Egg incubation and larval rearing were performed in 500 L tanks (one tank per duplicate), connected to closed recirculation systems, at an initial stocking density of 100 eggs L^{-1}. Until 20 days post-hatching (dph) larvae were fed on enriched rotifers (< 20 dph, DHA Protein Selco, INVE) in the presence of background phytoplankton (*Chlorella* sp., 300–600 × 10^3 cells mL^{-1}). After 12 dph Artemia instar I (12–20 dph, AF grade, INVE) and instar II (16–65 dph, EG grade, INVE) enriched nauplli (DHA Selco, INVE) were additionally provided to the larvae. Weaning to inert feed (O-range, INVE) started at 23 dph. During the larval rearing phase (11–55 dpf) water oxygen saturation was 7.6 ± 0.3 to 7.7 ± 0.4 mg/L^{-1} and pH 8.3 ± 0.1 to 8.4 ± 0.1 (Table S1). During the pre-growing period (56–98 dpf) fish were reared in a flow-through water-renewal system. Oxygen saturation was 6.5 ± 0.4 to 6.7 ± 0.3 mg/L^{-1} and pH 7.5 ± 0.1 (Table S2). Salinity (35‰) and photoperiod (12 L:12D) were constant during the entire experimental period. The same water source (borehole seawater) was used for the entire rearing period (1–98 dph). Fish rearing was performed at the Institute of Marine Biology, Biotechnology and Aquaculture (HCMR).

2.2 | Skeletal Analysis

To examine the development of skeletal abnormalities in the different experimental populations, fish samples were taken 1 week before the fish transfer to the pre-growing tanks (49 dpf, ca 15 mm SL), as well as three (68 dpf, ca 21 mm SL) and seven (98 dpf, ca 40 mm SL) weeks later (Table S3). Samples consisted of 85–101 fish, which were randomly taken from each experimental population (Table S3). All samples were anaesthetised (ethylenglycol-monophenylether, 0.2–0.5 mL L^{-1}) and fixed in 5% phosphate buffered formalin (pH = 7.2). The larval samples (49 and 68 dpf) were stained for bone and cartilage (Walker and Kimmel 2007). Following their fixation, juvenile samples (98 dpf) were preserved in 70% ethanol. SL measurements were performed post-staining or post-fixation for the larval or juvenile samples, respectively.

All experimental groups were examined for the presence of skeletal abnormalities at ca 15 mm SL, when most skeletal elements are expected to have formed in this species (Marino et al. 1993; Gluckmann et al. 1999; Fragkoulis et al. 2017). The samples of 21 and 40 mm SL were examined only for fin-ray loss. Differences in the frequency of morphological abnormalities among the different treatments were tested using *G*-test (Sokal and Rohlf 1981).

2.3 | Scanning Electron Microscopy

Ethanol preserved juvenile samples were dehydrated in an ascending alcohol series. Caudal fins were cut at their base, mounted on stubs, sputter-coated with gold palladium and examined using a JEOL JSM-6390LV scanning electronic

microscope at 15 kV at the Electron Microscopy Laboratory of the University of Crete.

2.4 | Gene Expression Analysis

At 69 dpf (ca 21 mm SL), a random sample of ca 15–20 specimens per experimental group was taken, anaesthetised (ethylenglycol-monophenylether, 0.2–0.5 mL L^{-1}), photographed under a stereoscopic microscope (Olympus SZ61, Lumenera Camera) and preserved in methanol, at −20°C. To test whether fin damage was linked with altered expression of genes involved in bone formation and resorption, the expression levels of genes implicated in the maturation of osteoblasts and osteoclasts and their activity were determined with RT-real time PCR.

A total of 30 (15 normal and 15 RSS) individual samples of the caudal region (caudal peduncle and fin) from European sea bass juveniles were collected and stored in methanol at −20°C until RNA extraction. Total RNA was extracted using E.Z.N.A. Total RNA Kit I (Omega) and was DNAse treated with the DNA-free DNA Removal Kit (Invitrogen) to remove traces of genomic DNA. For the cDNA synthesis, 200 ng of DNAse treated total RNA was reverse transcribed to cDNA using the High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems). cDNA was stored at −20°C until further use.

The expression of five target genes, acid phosphatase 5a (*acp5a*), metalloprotease 13a (*mmp13a*), osterix (*sp7*), osteocalcin1 (*bglap*) and osteoclacin2 (*bglapL*), was measured in reaction of 20 μL final volume using KAPA SYBR FAST qPCR Master Mix (2X) Kit (Kapa Biosystems) and the appropriate concentration of each transcript specific set of primers (Table 1). Primers were designed using the Primer3 (v.0.4.0) (Untergasser et al. 2012) and Beacon Designer software. Amplification cycle was 5 min at 95°C, followed by 40 cycles of 95°C for 20 s and 60°C for 20 s, followed by the dissociation curve step to verify for a single product amplification. Each reaction was performed in duplicate. A standard curve using a dilution series (1:5, 1:10, 1:20, 1:40, 1:100, 1:200) from pooled cDNA was constructed to estimate for amplification efficiency (Table 1). Three housekeeping genes (*fau*, *actb, rpl13*) were tested and assessed using the geNorm (Vandesompele et al. 2002) algorithm and the data was normalised using the geometric mean of the two most stably expressed housekeeping genes (*actb* and *rpl13a*). Expression data failed to meet the homogeneity of variances assumption and were analysed by using the non-parametric Kruskal–Wallis test ($\alpha = 0.05$).

3 | Results

3.1 | Ontogeny and Morphology of Ray-Resorption Syndrome (RSS)

At 49 dpf (ca 15 mm SL, Table S3) all experimental groups presented abnormally elongated lower jaw (Figure 1), at relatively high frequencies (27% ± 1%, 26% ± 17% and 16% ± 0% in the 14°C, 17°C and 20°C group, respectively). Differences in jaw abnormality frequencies among the experimental groups were not statistically significant ($p > 0.05$). At this stage, all fins presented normally developed rays and internal supporting elements (Figure 2A–C).

Two weeks after fish transfer to the weaning tanks (68 dpf, ca 21 mm SL, Table S3) all populations presented extensive ray loss or fracture (Figure 2D–G). Ray loss was present on the dorsal (55%–84%), pelvic (27%–53%) and anal (7%–17%) fins (Figure 3A,C,D). At this stage, caudal (Figure 3B) and pectoral fins did not present any missing rays. Fish microscopical examination revealed the presence of multiple non-mineralised areas, like typical resorption lacunae, scattered along the lepidotrichia of all the fins (Figure 2F–H). In the case of hard spines,

TABLE 1 | Primers and efficiencies used for qPCR per gene.

Gene id	Gene name	FW (RV)	Efficiency
ENSDLAG00005000664	*fau*	CTTCGTGAATGTTGTGCC (ACTGATGGATGGTGATGA)	99.6
ENSDLAG00005028561	*actb*	AAGCAGGAGTATGATGAGTC (GAAGTTGTTGGGCGTTTG)	101.7
ENSDLAG00005000624	*rpl13a*	GAAGGCATCAACATCTCC (CTCTGAAGTGGTAAGGTC)	101.7
ENSDLAG00005012618	*sp7*	GGTAGGGAATGTAATTGAAGACGC (TTTACTGGAGTAGTGCTGCCG)	99.9
ENSDLAG00005008130	*mmp13a*	AGAGTGTGGGCTTTCAGT (ACATCATAGAGGGCAGCA)	98.6
ENSDLAG00005014439	*acp5a*	GTAGGAGACTGGGGTGGAGT (GAAGTTATCGCCAAGGG)	98.3
ENSDLAG00005012194	*bglapL*	CTGGCTTCTGTTCTCCTG (TCACAGGCGATGCTCAACTC)	100
ENSDLAG00005013967	*bglap*	GCTGCTGGAGACTTATCCCT (GGTGTAGGCGGCAATGATTC)	99.8

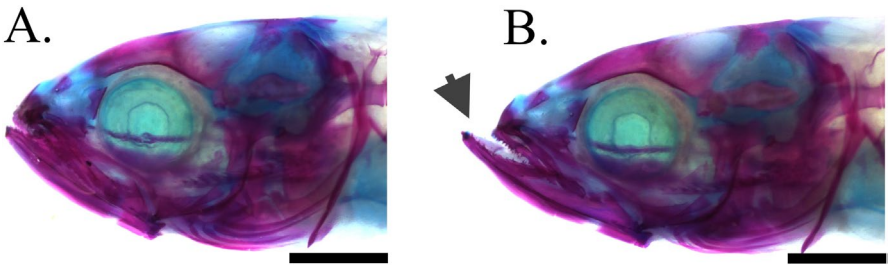

FIGURE 1 | Normal (A) and abnormally elongated (B) lower jaw in seabass larvae (49 dpf age, ca 15 mm SL). Scale bars equal to 1 mm.

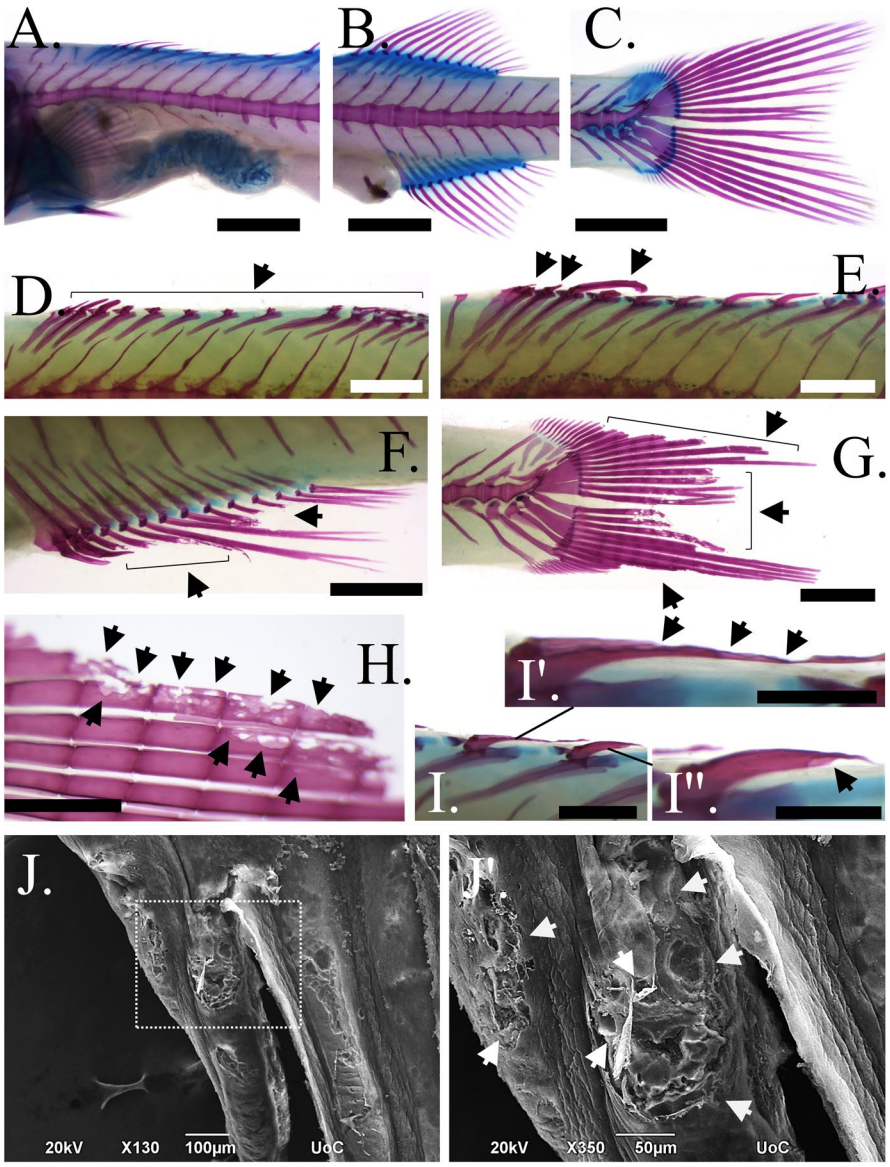

FIGURE 2 | Variability of ray resorption syndrome (RRS) in the examined samples. (A–C), normal fin anatomy at the middle of metamorphosis (ca 15 mm SL, 49 dpf). (D–I″), fins with abnormal rays (arrows) at the end of metamorphosis (ca 21 mm SL, 68 dpf). (D, E), dorsal fin. (F), anal fin. (G), caudal fin. (H, I), magnified rays of the caudal and dorsal fin respectively, with resorption lacunae (arrows). (I′, I″), insets of figure I, showing in detail the areas with bone resorption (arrows). (J). Examination of RSS by scanning electron microscopy (SEM) at the juvenile stage (ca 40 mm SL, 98 dpf). Photograph shows part of a caudal-fin with RSS. (J′). Inset of figure J shows multiple resorption lacunae (arrows). Scale bars equal to 1 mm (A-G) or 2 mm (H-I″).

bone resorption was evident in the form of irregular weakening (Figure 2I–2I″). The highest rates of ray-resorption syndrome (RSS) were observed on the caudal (83%–86%) and dorsal (71%–74%) fins and the lowest on the pectoral fins (5%–18%) (Figure 3E). The frequency of RSS was significantly differentiated among the three fish groups in the case of the anal, pelvic

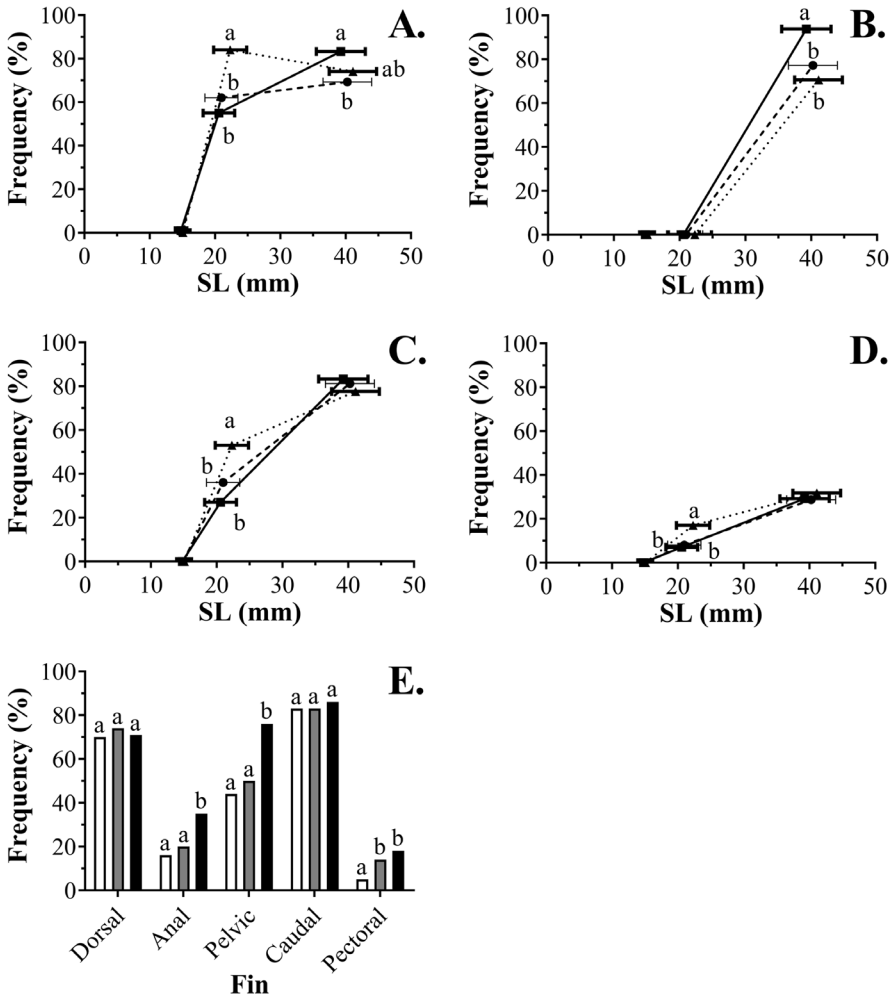

FIGURE 3 | (A-D), change of ray-absence frequency on the dorsal (A), caudal (B) pelvic (C) and anal (D) fins with the growth of fish standard length (SL). Circles, squares and triangles symbols indicate 14°C, 17°C and 20°C developmental temperature, respectively. Horizontal error bars equal to 1 SD. (E), Frequency of ray resorption syndrome (RRS) on the different fins, at the end of metamorphosis (ca 21 mm SL, 68 dpf). Open, grey and black bars correspond to 14°C, 17°C and 20°C developmental temperature, respectively. Different letters indicate significant differences in the frequency between the different thermal regimes (*G*-test, $p < 0.05$).

and pectoral fin, but not in the case of fins with the highest RSS frequency (dorsal and caudal) (Figure 3E).

At 98 dpf (ca 40 mm SL, Table S3), an extensive loss of rays was present on the fins of all fish groups (Figure 4A–F). The highest rates of ray loss were observed on the dorsal (74%–83%, Figure 3A), caudal (71%–94%, Figure 3B) and pelvic fins (78%–83%, Figure 3C). Anal (29%–32%, Figure 3D) and pectoral (1%–2%) fins presented the lowest rates of ray loss. Scanning electron microscopy revealed the presence of bone resorption areas on the surface of the remaining rays. Resorption areas were characterised by multiple lacunae of different size (Figure 2J–J').

3.2 | Gene Expression Analysis of RRS

Specimens were categorised according to their caudal-fin morphology into the normal group (Figure S1A) or the group with damaged fin rays (Figure S1B,C). Caudal fin was selected to test the association of gene expression with ray resorption (RSS) because it exhibited the highest rates of RSS at 68 dph (Figure 3E). Analysis was performed on a piece of tissue that was removed from each specimen, consisting of the caudal-fin and the caudal peduncle.

The analysis of established gene markers of osteoblast maturation (*sp7, bglap, bglapL*) revealed significantly higher expression of *bglapL* in RSS specimens in comparison with normal (Figure 5A). Genome search resulted in the discovery of two genes encoding European sea bass osteocalcin, *bglap* and *bglapL*. *bglap* was consistently expressed at higher levels than *bglapL*; however, no differences were observed between normal and RSS specimens (Figure 5B). Similarly, *sp7* expression levels were not significantly affected by RSS (Figure 5C). Osteoclasts hold a dynamic role in bone formation and resorption and *acp5a*, a gene marker of osteoclast maturation, was significantly upregulated in RSS specimens (Figure 5D). One gene marker (*mmp13a*) associated with degradation of extracellular matrix was also evaluated. Interestingly, *mmp13a* that encodes for a collagenase exhibited significantly increased expression in RSS specimens (Figure 5E).

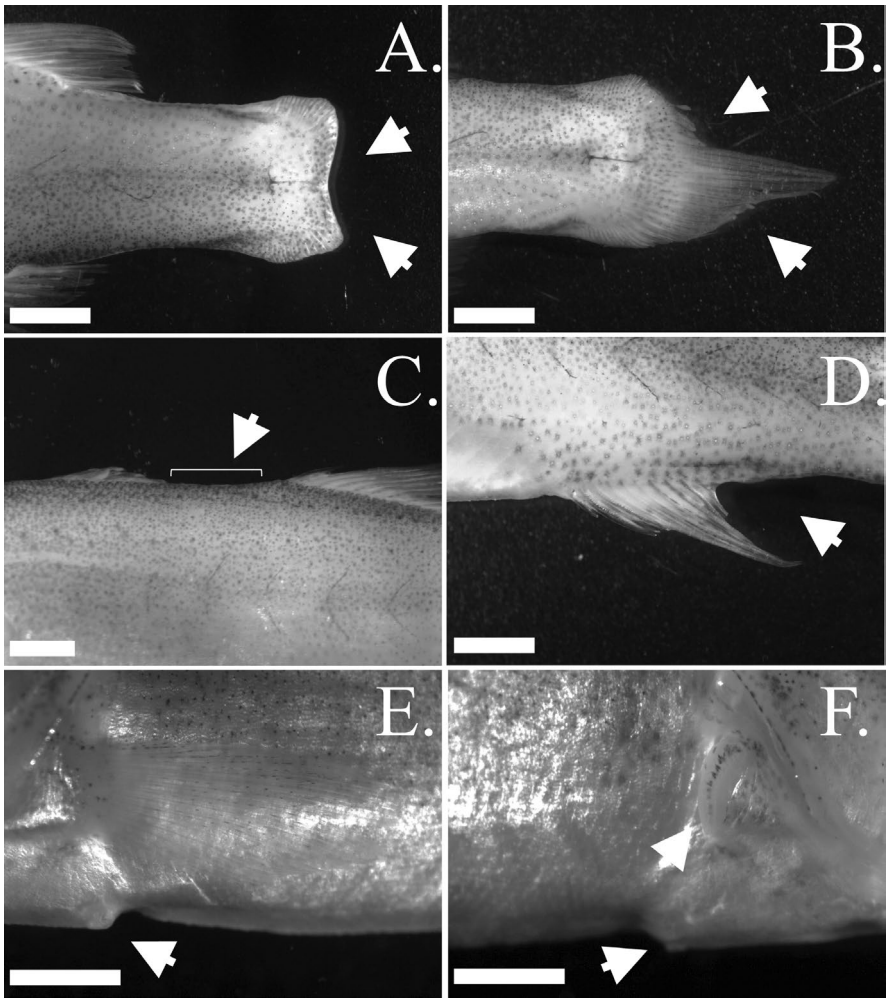

FIGURE 4 | Partial to complete absence of fins in seabass at the juvenile stage (ca 40 mm SL, 98 dpf). (A, B), caudal fin. (C), dorsal fin. (D), anal fin. (E), pelvic fins. (F), pelvic and pectoral fins. Arrows indicate the missing fin parts. Scale bars equal to 2 mm.

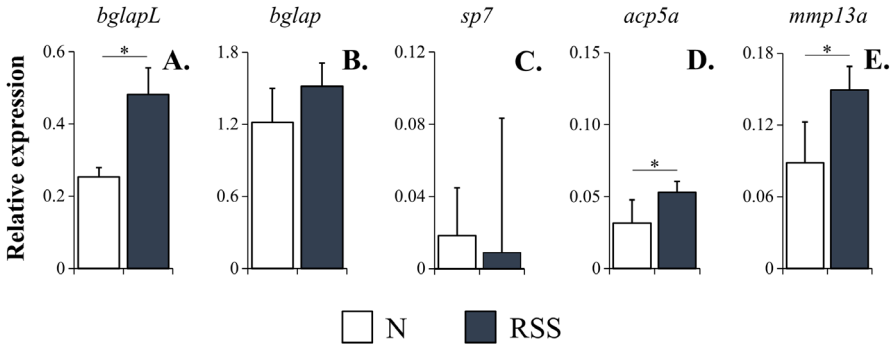

FIGURE 5 | Relative gene expression levels (mRNA) in European seabass specimens with normal (N) or damaged fin rays (RSS). (A), osteocalcin2 (*bglapL*). (B), osteocalcin1 (*bglap*). (C), osterix (*sp7*). (D), acid phosphatase 5a (*acp5a*). (E), metalloprotease 13a (*mmp13a*). Asterisks indicate significant differences between N and RSS fish ($p < 0.05$). Error bars equal to 1 SE.

4 | Discussion

Ray-resorption syndrome (RSS) was first described in samples of *S. aurata* metamorphosing larvae without, however, been related with fin erosion (Kourkouta et al. 2022). In the present study, RSS was recorded to develop in European seabass during the late metamorphosis period, and up to the end of metamorphosis resulted in extensive fin erosion, due to fraying of the weakened fin rays. As in the case of *S. aurata* (Kourkouta et al. 2022), RSS was not linked with abnormalities of the fin-supporting elements and developed after the completion of fin skeleton development. Our results showed that the caudal, dorsal and pelvic fins were the most prone to RSS and the following fin erosion in metamorphosing larvae and early juveniles. This finding agrees with the existing literature, which shows that different fins may be differentially prone to fin damage. In Salmonids for

example, dorsal and pectorals are generally more prone than the rest fins, whereas caudal fin is most affected in *Solea solea* and the paired fins in walleye (reviewed by Ellis et al. 2008). Following Person-Le Ruyet and Le Bayon (2009), caudal and dorsal fins were the most eroded in European seabass during the on-growing period. This study suggested that differences in fin vulnerability to fin erosion might be indicative of differences in the activity of each fin during fish movement (e.g., caudal fin is highly active for propulsion, dorsal fin for manoeuvring and stabilisation) and on the loads acting on them by the water. This hypothesis, however, cannot explain the differences in RSS frequency among the different fins (present study).

The analysis of established gene markers of osteoblast maturation (*sp7, bglap, bglapL*, Knopf et al. 2011) revealed significantly higher expression of *bglapL* in RSS specimens in comparison with normal. Osteoblast signalling is a prerequisite for the differentiation of osteoclasts, which hold a dynamic role in bone formation and resorption. The tartrate-resistant acid phosphatase (*acp5a*) is a molecule required for its resorptive function (Alesi, Charles, and Nakamura 2020). Indeed, *acp5a* was significantly upregulated in RSS specimens. Interestingly, *mmp13a* that encodes for a collagenase implicated in the remodelling of extracellular matrix (Li, Zhang, and Akimenko 2020), was also significantly upregulated in RSS specimens. The gene landscape indicates an enhanced osteoclast activity and matrix remodelling, possibly driven by the signalling of osteoblasts, thus further supporting our hypothesis on the link between the observed abnormal phenotype and bone resorption. A variety of nutritional imbalances may increase bone resorption in fish (e.g., Vitamin K, dietary phosphorus, reviewed by Lall and Lewis-McCrea 2007) or are shown as critical for fin erosion in fish (e.g., PUFAs, vitamin C, lysine, reviewed by Latremouille 2003). In the present study, fish were fed standard commercial diets, which were also used during the period prior to RSS development.

RSS was first observed 2 weeks after fish transfer to the weaning tanks, which was associated with the use of seawater of lower pH than before (7.5 vs. 8.5–8.6 mean pH, Tables S1 and S2). Low pH conditions during the weaning period were caused by elevated levels of dissolved CO_2 in the borehole water used (Tsertou et al. 2022) in the flow-through water-renewal system of the weaning tanks. During the larval rearing phase, the water maturation during biological filtration and aeration in the closed recirculation system removed the excess of CO_2 and increased the water pH. Prolonged exposure of fish to elevated CO_2 concentrations leads to an increase in blood CO_2 levels (hypercapnia) and a decrease in blood pH, ultimately causing respiratory acidosis (Eddy et al. 1977; Ultsch 1996). To counteract acidosis, fish enhance plasma bicarbonate levels and excrete phosphate through the kidneys (Lloyd and White 1967) and may also mobilise ions from bone tissue (Storset, Åsgård, and Bæverfjord 1997). In Atlantic salmon smolts, the exposure to high CO_2 levels increased the volume of vertebral trabeculae and the rate of bone remodelling at the end of the freshwater period (Gil Martens et al. 2006). This increased bone volume might be based on a mobilisation of Ca/P deposits from scales, which are the primary source of calcium under conditions of increased calcium demands (Metz et al. 2014). In the present study, RSS in European seabass appeared during the late metamorphosis period, before the squamation completement (end of metamorphosis, ca 28–30 mm TL, own unpublished data). Under hypercapnia conditions and lack of fully developed scales in European seabass larvae, it might be hypothesised that RSS is induced by phosphate excretion and ion mobilisation from the fin rays.

Fin erosion may be associated with bacterial infections, even though the latter may be a secondary infection and not necessarily the primary cause of fin damage (reviewed by Ellis et al. 2008, Latremouille 2003). For example, frayed fins are a primary symptom of tenacibaculosis across various fish species (Avendaño-Herrera, Toranzo, and Magariños 2006), including European seabass (Gourzioti et al. 2018). In the present study, although no ulcerative skin lesions or other external clinical signs were observed on the examined fish, the involvement of microbial agents on the development of RSS cannot be excluded. To our knowledge, however, no study exists so far on the link between RSS and infectious diseases.

Fish have a remarkable capacity to regenerate their fins following erosion (Noble, Mizusawa, and Tabata 2007) or experimental partial ablation (e.g., Uemoto, Abe, and Tamura 2020). However, proximal amputation near the base of the rays often leads to non-regeneration or incomplete regeneration (Shao et al. 2009). In the present study, a complete loss of rays was observed in many juveniles, raising the question of whether the missing fin rays could regenerate during the subsequent growing period.

5 | Conclusions

In the present study, we documented the presence of ray-resorption syndrome (RSS) in metamorphosing European seabass larvae. Up to the early juvenile period, RSS led to severe ray loss on all the fins. Ray-bone resorption was linked with altered expression of genes involved in osteoclast activity and matrix remodelling. To our knowledge, this, along with haemal lordosis (e.g., Sfakianakis et al. 2006), is the second post-metamorphic abnormality that may develop in reared European seabass. RSS should not be confused with the partial to complete absence of fins that develops during the larval and early metamorphosis period (e.g., caudal-fin loss, Haga, Takeuchi, and Seikai 2002; saddleback syndrome, Fragkoulis et al. 2017). From an applied perspective, this post-metamorphic loss of fins strongly suggests the need to modify quality control schemes in European seabass hatcheries, including additional fish examinations from metamorphosis to the end of the pre-growing phase.

Author Contributions

Chara Kourkouta: investigation, formal analysis, visualization, writing – review and editing. **Andreas Tsipourlianos:** investigation, formal analysis, writing – review and editing. **Nikos Papandroulakis:** resources, writing – review and editing, investigation, funding acquisition. **Katerina A. Moutou:** investigation, formal analysis, writing – review and editing, writing – original draft, funding acquisition, supervision. **George Koumoundouros:** conceptualization, writing – review and editing, writing – original draft, formal analysis, supervision, funding acquisition, visualization.

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Ethics Statement

The experimental protocol was approved by the Veterinarian Authority of the Region of Crete with the 255332/29-11-2017 document. Animal experiments were carried out in accordance with the EU Directive 2010/63/EU.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.