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RESEARCH ARTICLE

The role of starter diets in the development of skeletal abnormalities in zebrafish *Danio rerio* (Hamilton, 1822)

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Abstract

Fish skeletal development has long been correlated with nutritional factors. Lack of zebrafish nutritional standardization, especially during the early stages, decreases the reproducibility of the conducted research. The present study represents an evaluation of four commercial (A, D, zebrafish specific; B, generic for freshwater larvae; C, specific for marine fish larvae) and one experimental (Ctrl) early diets on zebrafish skeletal development. Skeletal abnormalities rates in the different experimental groups were assessed at the end of the larval period (20 days post-fertilization, dpf) and after a swimming challenge test (SCT, 20–24 dpf). At 20 dpf, results revealed a significant effect of diet on the rate of caudal-peduncle scoliosis and gill-cover abnormalities, which were relatively elevated in B and C groups. SCT results focused on swimming-induced lordosis, which was comparatively elevated in diets C and D (83% ± 7% and 75% ± 10%, respectively, vs. 52% ± 18% in diet A). No significant effects of dry diets were observed on the survival and growth rate of zebrafish. Results are discussed with respect to the deferential diet composition between the groups and the species requirements. A potential nutritional control of haemal lordosis in finfish aquaculture is suggested.

KEYWORDS
abnormalities, finfish larvae, lordosis, nutrition, scoliosis, skeleton

1 | INTRODUCTION

Targeting, nowadays, fast growth, high survival rates, improved reproductive performance and production cost efficiency has set aside the importance of fish skeletal integrity. The rate of skeletal abnormalities is a valuable welfare index for reared and laboratory fish, reflecting the appropriateness of the practices applied during early life stages (Koumoundouros, 2010; Printzi, Kourkouta, et al., 2021). Embryonic and larval periods have been highlighted as critical for developing a normal skeletal pattern (Georga et al., 2011; Koumoundouros et al., 1997). Skeletal abnormalities can alter fish performance (e.g., growth, survival) and their development has been correlated with several abiotic stimuli (e.g., temperature, nutrition, etc. Boglione et al., 2013; Eissa et al., 2021).

Zebrafish is currently highlighted as a model species for vertebral skeletogenesis and skeletal deformities research (Luderman et al., 2017; Printzi, Fragkoulis, et al., 2021; Witten et al., 2017), sharing a similar bone matrix and bone cellular function (osteocytes, chondrocytes) with mammals (Dietrich et al., 2021). Although the significant effect of husbandry conditions on fish skeletal development is well-documented (Cahu, Zambonino Infante, & Takeuchi, 2003), our knowledge on the effect of dietary regimes on zebrafish skeletal

elements remains restricted. Single nutrient elements studies (e.g., phosphorus or phospholipids; Costa et al., 2018; Cotti et al., 2020; Martins et al., 2020) or whole-feed composition studies effect on the frequency of induced skeletal deformities (Martins et al., 2019; Printzi, Kourkouta, et al., 2021) represent the approaches to date. Hence, lack of nutritional standardization (nutrition and regime) for a model species largely used in different facilities for biomedicine, toxicology and aquaculture purposes, can increase the variability of the conducted research (Lawrence, 2016). Taking into account the direct nutritional impact on both the physiological (development, health, diseases) and molecular level of individuals, a need for a well-defined diet of reproducible quality exists (Watts et al., 2016).

Being still a controversial factor, live food inclusion, either exclusively or partially during early stages of fish, has been correlated with increased larval growth and survival rates (Best et al., 2010; Kolkovski, 2013; Lawrence et al., 2015). Several live preys, such as *Artemia* nauplii (*Artemia* sp.), paramecia (*Paramecium* sp.) and rotifers (*Brachionus* sp.) have been utilized during zebrafish early stages, with variable results in terms of fish survival and growth (Best et al., 2010; Lawrence et al., 2015; Samaee et al., 2021). In either case, live food incorporation in the larvae dietary regime cannot guarantee an adequate nutrient supply that highlights the increasing need of transition to specialized inert diets.

Many of the diets used for zebrafish rearing over the years have been originally designed for commercial marine or ornamental species (Watts & D'Abramo, 2021). Research on formulation of zebrafish microdiets for successful early weaning has been focused on replacing live-food during the first days of exogenous feeding (Carvalho et al., 2006; Goolish et al., 1999). Simultaneously, during the early larval stages, several key factors have been reported as critical such as the applied regime (Lawrence, 2007; Monteiro et al., 2018; Siccardi et al., 2009), the transition rate from live to dry feed, the timing and frequency of food provision (Kaushik et al., 2011; Lawrence et al., 2012) and the water quality (Hammer, 2020). Studies utilizing the commercially available diets for the species from mouth opening resulted in lower growth and survival levels were compared to live-food inclusion (Carvalho et al., 2006; Goolish et al., 1999). Hence, recent trials with an early transition strategy to dry feeds are reported to be promising in terms of increased growth and survival rates (Farias & Certal, 2016) while ensuring low skeletal deformities presence (Martins et al., 2019; Printzi, Kourkouta, et al., 2021). However, according to our knowledge, no study exists on the evaluation of the commercially available feeds on the skeletal development of zebrafish.

This study represents an evaluation of four commercial larval feeds (two for zebrafish, one generic for freshwater larvae and one for marine fish larvae) on zebrafish skeletal development. One experimental starter diet, designed according to a previously reported as optimal for marine larvae, was also included. Evaluation took place (a) at the end of the larval period based on the frequency of skeletal abnormalities and (b) at the early juvenile stage to assess the integrity of the vertebral column against increased exercise conditions. Simultaneously, performance traits as growth and survival were followed.

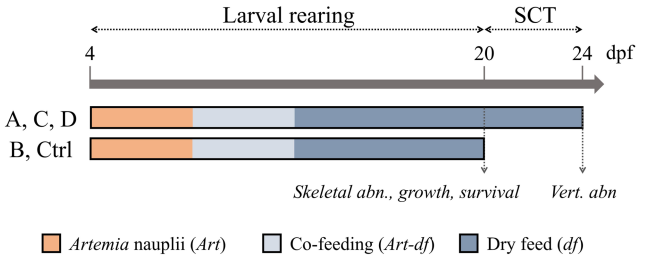

FIGURE 1 Experimental design. Larvae were reared under common abiotic conditions, with the use of five different dry feeds (A–D, Ctrl). All trials were performed in triplicate. At 20 days post-fertilization (dpf) all groups were examined for fish size (TL), survival rate and the development of skeletal abnormalities. A, C and D groups were additionally subjected to a swimming challenge test (SCT) of 4 days (20–24 dpf) duration and examined for the presence of vertebral abnormalities. Feed quantities are given in Table 1.

2 | MATERIALS AND METHODS

To evaluate the effect of the starter diets on zebrafish skeletal development, the experimental design depicted in Figure 1 was applied. Feeding regimes included a common 4-day period of exclusive *Artemia* nauplii provision, followed by a 4-day co-feeding before the total switch on the applicable dry feed of each group (Table 1). Skeletal development was assessed based on the skeletal abnormalities rate at the end of the larval rearing period (20 dpf, days post-fertilization) and based on the frequency of exercise-induced lordosis (haemal lordosis) following a 4-day swimming challenge test (SCT, 20–24 dpf). All trials were performed in three independent replicates.

2.1 | Animal rearing

For each replicate, fertilized eggs were acquired from a common 250 wild-type zebrafish population (F WT2 F17; Wageningen Agricultural University, Wageningen, the Netherlands) and randomly divided in five experimental groups (250 embryos per experimental replicate and diet). Egg incubation and larval rearing of each experimental replicate took place in five cubic net pens of 4.5 L volume each and 100 μm mesh size, positioned into a common aquarium of 60 L volume, connected to a biological filter. Abiotic measurements were taken on a daily (temperature, pH, conductivity, oxygen saturation) or weekly basis (nitrogen compounds) (Table 2).

Following swim bladder inflation (4 dpf), all groups were initially fed with newly hatched *Artemia* nauplii (4–7 dpf, *Artemia* AF; INVE, Dendermonde, Belgium), then with a mix of *Artemia* nauplii and dry feed (8–11 dpf), and finally with dry feed (>12 dph, Table 1). Of the four commercial diets tested, two are available for zebrafish larvae (A with crumble-size of minimum 50 μm and D of <100 μm minimum crumble-size), one is commercially available for freshwater fish larvae (B, 100 μm min crumble-size) and one for marine fish larvae (C, 80 μm min crumble-size). The control diet (Ctrl, 200–400 μm min

crumble-size range) is an experimental diet manufactured at Ifremer facilities (French National Institute for Marine Science, Plouzané, Brest, France), and similar to the best one used in Cahu, Infante, and Barbosa (2003).

Breeders were kept in a 120-L aquarium connected to a closed recirculating system at 28.0°C (±0.5), 500–700 µS cm^{-1} conductivity, 7.0–7.5 pH, 85%–95% oxygen saturation (at the applied temperature the 100% oxygen saturation corresponds to 7.8 mg/L) and 14/10 h light/dark photoperiod. Their feeding included a commercial dry diet (Zebrafeed, Sparos), with an *Artemia* nauplii supplementation twice a week.

2.2 | Skeletal abnormalities, growth and survival

At 20 dpf, a random sample of 50 individuals from each group and replicate was acquired to estimate skeletal abnormalities and fish size. Individuals were euthanatized (overdose of 2-phenoxyethanol, 0.3–0.5 mL L^{-1}), fixed in 5% phosphate-buffered formalin (pH = 7.2) and stained for cartilage and mineralised bone (Walker & Kimmel, 2007). Stained samples were photographed under a stereo-microscope (Olympus SZ61) and measured for total length (TL) using tpsDig2 software (Rohlf, 2010). Cranial, vertebral and fin abnormalities were examined following the classification methodology and terminology of Koumoundouros (2010). When an individual presented more than one abnormality, multiple abnormality scores were recorded. Following Harder (1975), the terms prehaemal and haemal were used to describe the vertebrae with and without a haemal spine, respectively (corresponding to the pre-caudal and caudal vertebrae of Bird & Mabee, 2003).

Survival rates were calculated on the counts of fish in each net-pen at 20 dpf.

TABLE 1 Feed quantity (per experimental group) and feeding frequency during the larval rearing period.

Age (dpf)	Feed type	Quantity (per meal)	Daily meals
4–7	*Artemia* nauplii (ind)	4000	5
	Dry feed (mg)	–	–
8–9	*Artemia* nauplii (ind)	6000	3
	Dry feed (mg)	50	2
10–11	*Artemia* nauplii (ind)	6000	2
	Dry feed (mg)	63	3
12–16	*Artemia* nauplii (ind)	–	–
	Dry feed (mg)	75	5
17–20	*Artemia* nauplii (ind)	–	–
	Dry feed (mg)	100	5

2.3 | Swimming challenge test (SCT)

After abnormalities sampling (20 dpf), 20 juveniles (11–12 mm total length, TL) without any gross abnormalities (i.e., lordosis, scoliosis) were selected from each of the A, C and D groups for SCT. The selection of the groups for the SCT aimed at the evaluation of the two species-specific diets (A, D), against one that presented altered skeletogenesis during the larval stage (C). Following Printzi, Fragkoulis, et al. (2021), zebrafish were subjected to 4 days of continuous swimming (24 h per day) against a water velocity of 8.0 TL s^{-1} (total length per sec). Fish were fed three times per day during short water-flow cut offs for 15 min. Three swimming tunnels (70 cm length, 10 cm depth, 5 cm width) were utilized in order to perform the swimming trials of each replicate simultaneously. These custom-designed swimming apparatuses are closed recirculating systems, which maintain the laminar flow using an external stack of flow tubes and adjustable valves (Printzi, Fragkoulis, et al., 2021). Abiotic conditions during the SCT were maintained at 28.0°C (±0.5°C), 500–700 µS cm^{-1} conductivity, 7.0–7.5 pH, >90% oxygen saturation and 14/10 h light/dark photoperiod through the closed recirculation systems.

At the end of the trials, individuals were euthanatized (2-phenoxyethanol, 0.3–0.5 mL L^{-1}), fixed in 5% phosphate-buffered formalin and stained for cartilage and mineralized bone (Walker & Kimmel, 2007). Stained samples were photographed under a stereo-microscope (Olympus SZ61) and examined for the presence of haemal lordosis (Printzi, Fragkoulis, et al., 2021).

2.4 | Feed proximate analysis

Two duplicates of 0.10–0.11 g of each feed (freeze-dried *Artemia*, A, B, C, D, Ctrl) were acquired for their chemical analyses. Ash (7 h

TABLE 2 Abiotic parameters during zebrafish larval rearing (mean ± SD).

Water parameters	Replicate 1	Replicate 2	Replicate 3
Temperature (°C)[a]	27.3 ± 0.4	27.3 ± 0.2	27.6 ± 0.2
Oxygen saturation (%)	93.1 ± 2.1	95.1 ± 1.4	94.3 ± 3.4
Conductivity (µS/cm)	471.6 ± 16.8	485.2 ± 28.2	596.2 ± 29.9
pH	7.9 ± 0.2	8.2 ± 0.1	8.3 ± 0.1
Ammonia mg/L (ppm)	<0.01	<0.01	<0.01
Nitrite mg/L (ppm)	0.012 ± 0.01	0.035 ± 0.03	0.01 ± 0.004
Nitrate mg/L (ppm)	7.5 ± 3.54	7.5 ± 3.54	6.5 ± 2.12

[a]At the applied temperature, the 100% of oxygen saturation corresponds to 7.8 mg/L in fresh water.

at 550°C), crude fat (Folch et al., 1957), crude protein (the Kjeldahl method, $N \times 6.25$) and phospholipid and neutral lipid (Juaneda & Rocquelin, 1985) contents were evaluated (Table 3). The freeze-dried *Artemia* was collected after the nauplii hatching, to be consistent with the feeding procedures followed.

2.5 | Statistical analysis

The differences in abnormality rates among the experimental groups were tested by G-test (Sokal & Rohlf, 1981). The non-parametric Kruskal–Wallis and Mann–Whitney *U*-test were used to test the significance of the differences in total length (TL) and survival rate among the different groups. ANOVA was not used because the assumptions of normality or homogeneity of variances were not fulfilled (Sokal & Rohlf, 1981).

2.6 | Ethical statement

All the experimental procedures involving zebrafish were performed in accordance with Greek (PD 56/2013) and EU (Directive 63/2010) legislation for animal experimentation and welfare. All protocols are approved by the Animal Care Committee of the Biology Department of the University of Crete (Permit number: 47006/21).

3 | RESULTS

At the end of the larval rearing period, a variety of different abnormality types was present in the examined samples. Scoliosis of the caudal peduncle (Figure 2a), gill-cover wrinkling (Figure 2b) and abnormalities of the supporting elements of caudal-fin (Figure 2c) were the most frequent types, followed by prehaemal kyphosis, haemal lordosis (Figure 2d), abnormalities of vertebral processes and light jaw abnormalities (Figure 3a–g). Dry feed had a significant effect on the development of caudal-peduncle scoliosis and gill-cover wrinkling, both of which were comparatively elevated in B and C groups (Figure 3a,b). Rest abnormalities were not significantly affected by the dietary conditions tested (Figure 3c–g).

TABLE 3 Chemical composition (%) of the diets tested (mean values).

Feed	% DM	% Ash	% Proteins	% TL	% NL	% PL
Artemia	94.8	6.5	61.7	19.2	9.2	6.6
Control	94.0	12.0	54.7	16.7	4.7	11.7
A	93.9	13.9	66.8	18.5	4.0	8.8
B	93.9	12.0	63.5	12.6	5.7	4.6
C	93.2	14.0	67.3	16.8	6.8	5.5
D	91.0	12.7	67.7	13.0	4.0	6.3

Abbreviations: DM, dry matter; NL, neutral lipids; PL, phospholipids; TL, total lipids.

Fish exposure to SCT induced the development of haemal lordosis (Figure 2d) and caudal-peduncle scoliosis (Figure 2a). Tested diets significantly affected the swimming induced vertebral abnormalities. Compared with the A group, the C and D groups presented a significant increase in the total frequency of vertebral abnormalities (Figure 4a), which was mostly attributed to the development of haemal lordosis (Figure 4b). Starter diets did not have a significant effect on the development of caudal-peduncle scoliosis after the SCT (Figure 4c). Interestingly, approximately the 48%–67% of the lordotic fish simultaneously presented caudal-peduncle scoliosis (Figure 4b).

In all replicates, B and C diets presented smaller survival rate than the rest of the groups (Figure 5a). However, the effect of dietary regimes on mean survival rate was not proven significant ($p = .06$, Kruskal-Wallis, Figure 5b). With regard to fish growth, despite the significant effect of starter diets on fish TL at 18–20 dph (second and third experimental replicate), TL response to the dietary regimes was not repeated in the different replicates (Figure 5c).

According to the feed proximate analyses, the protein content of the diets ranged between 54.7% and 67.7%, whereas the total lipid content varied from 12.6% to 18.5%. Neutral lipids and phospholipids contents ranged between 4.0%–6.8% and 4.6%–11.7%, respectively, among the diets (Table 3).

4 | DISCUSSION

Nutrition is a key factor for the normal development of fish larvae. Currently, relevant literature targets primarily species of interest to aquaculture (Boglione et al., 2013; Lall & Kaushik, 2021), but rarely targets laboratory fish (Martins et al., 2019; Printzi, Fragkoulis, et al., 2021). Here, we examined the effect of five different starter diets on the development of skeletal abnormalities, growth and survival of zebrafish. Our results showed that dry feed has significant effects on the frequency of the caudal-peduncle scoliosis and gill-cover wrinkling at the end of metamorphosis, as well as on the response of juvenile vertebrae to increased swimming exercise. Although zebrafish larvae accepted well the dry feeds tested, the use of zebrafish-specific microdiets (i.e., A, D) substantially improved larval skeletal quality and survival rates when compared to the generic and the marine diets (i.e., B, C).

For a given species, different types of skeletal abnormalities may present different responses to the same causative factor. For example, in sea bass larvae lower levels of vitamin A minimized the jaw and hyoid deformities, whereas vertebral and fin elements had a better development under increased retinol levels (Mazurais et al., 2009). Such differences are suggested being related to the development of the different skeletal elements at different ontogenetic windows, as well as with ontogenetic shifts in environmental and nutritional preferences of fishes (Georgakopoulou et al., 2007; Mazurais et al., 2009). In the present study, caudal-peduncle scoliosis and gill-cover abnormalities were both elevated in the dietary groups B and C, whereas the rest abnormalities were unaffected by the tested

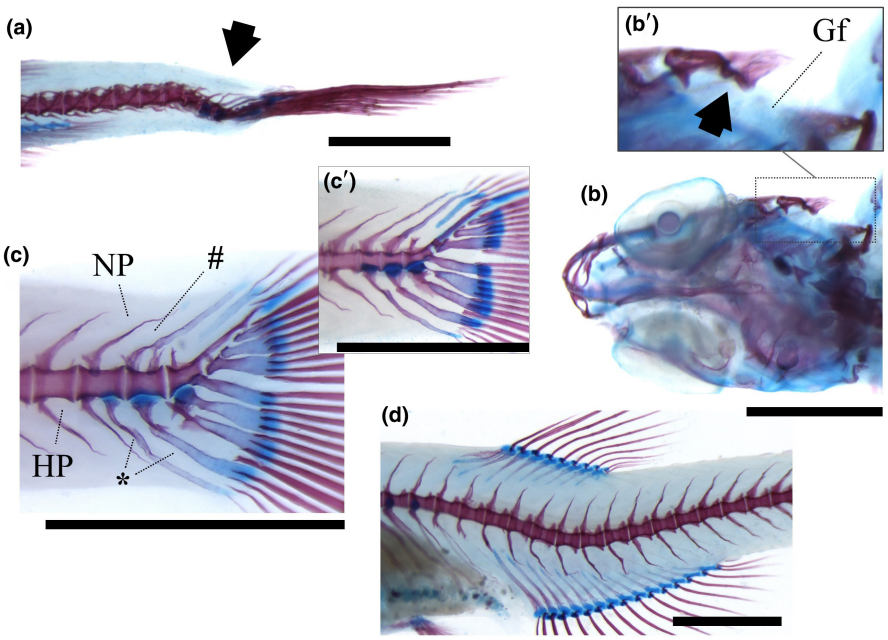

FIGURE 2 Main types of skeletal deformities developed in zebrafish larvae. (a) Scoliosis of the caudal-peduncle. (b) Abnormal gill cover. (b'). Magnification of the abnormal gill cover exposing the gill filaments (Gf). (c) Abnormalities of the caudal-fin skeleton, with extra formation of haemal (*) or neural (#) processes. (c'). Normal bone structure of the caudal fin. (d) Haemal lordosis. HP, haemal process; NP, neural process. Scale bars equal to 1 mm.

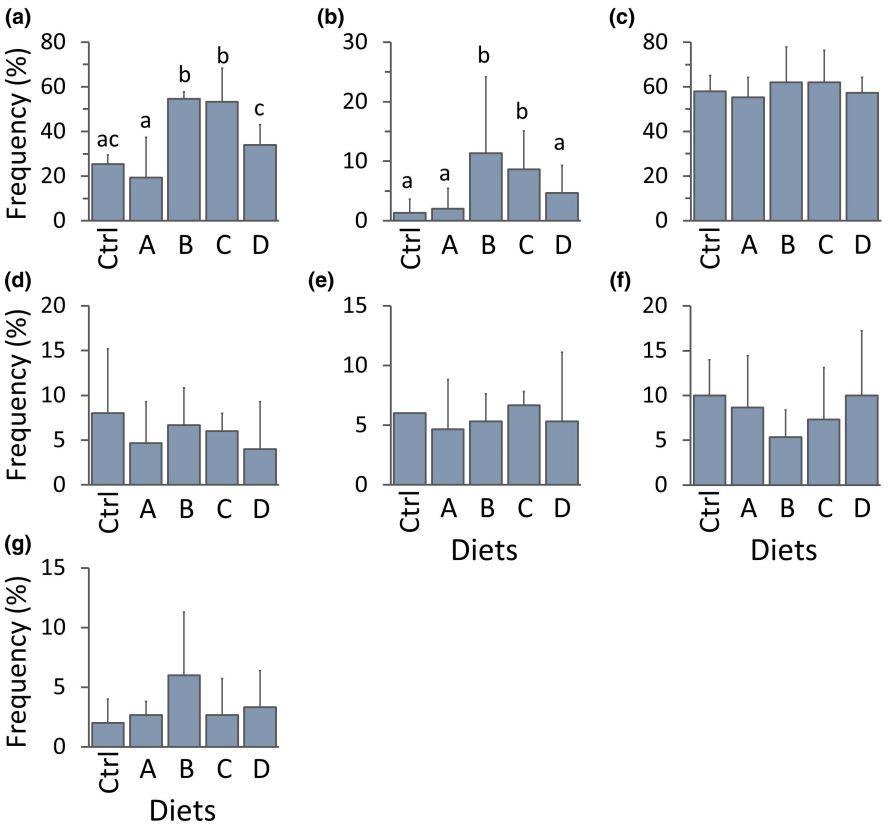

FIGURE 3 Effect of starter diets on the frequency of the main skeletal deformities observed in zebrafish at the end of larval rearing period (50 larvae per replicate, 3 replicates per diet, 18–20 dpf). (a) Scoliosis of the caudal-peduncle. (b) Gill-cover abnormalities. (c) Abnormalities of the caudal complex. (d) Prehaemal kyphosis. (e) Haemal lordosis. (f) Miscellaneous vertebral abnormalities. (g) Jaw abnormalities. Different letters indicate significant differences ($p < .05$, G-test). Error bars equal to 1 SD.

microdiets. Gill-cover abnormalities develop frequently in a variety of species, during the early larval period, due to the action of various factors (e.g., vitamin A, Fernández et al., 2008); water temperature (Georgakopoulou et al., 2010). In zebrafish, (Printzi, Kourkouta, et al., 2021) showed that the frequency of gill-cover abnormalities increases with the increase of *Artemia* replacement by microdiets. To our knowledge, caudal-peduncle scoliosis is reported at high rates only in laboratory zebrafish (present study, Martins et al., 2019; Printzi, Kourkouta, et al., 2021). Printzi, Kourkouta, et al. (2021) suggested that caudal-peduncle scoliosis may result from a relatively fast dietary shift from *Artemia* nauplii to microdiets. The results of the present study showed that under a standard *Artemia* replacement protocol, larval microdiets may significantly affect the rates of gill-cover abnormalities and caudal-peduncle scoliosis.

Haemal lordosis is a frequent vertebral abnormality in reared fish (including zebrafish), that develops mainly due to the intense

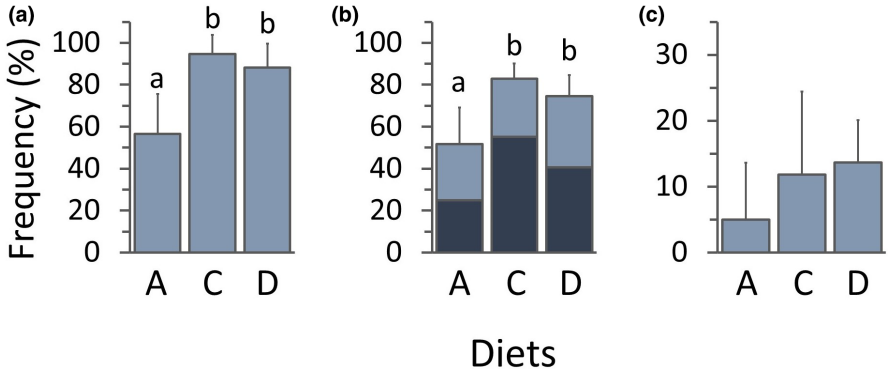

FIGURE 4 Effect of starter diets on the frequency of vertebral abnormalities in zebrafish, following a 4-day swimming challenge test (20 individuals per replicate, 3 replicates per diet). (a) Total abnormalities of the vertebral column. (b) Haemal lordosis. The dark area indicates the frequency of fish with additional development of caudal-peduncle scoliosis. (c) Caudal-peduncle scoliosis. Different letters indicate significant differences ($p < .05$, *G*-test). Error bars equal to 1 SD.

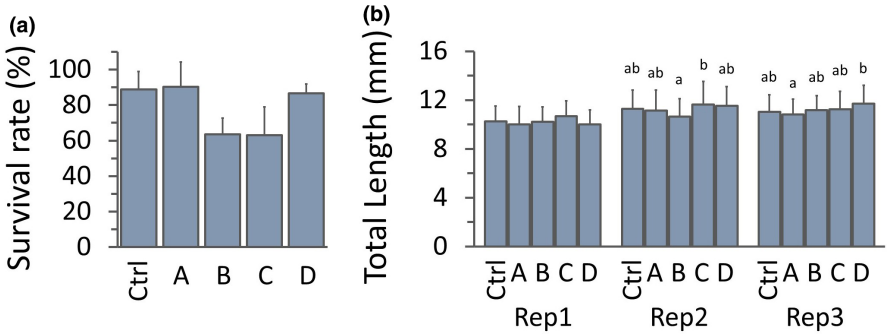

FIGURE 5 Effect of starter diets on the mean survival (a) and total length (b) 50 larvae per diet per replicate) of zebrafish at the end of larval rearing period (18–20 dpf). Rep1-Rep3, Replicate 1–3, respectively. Different letters indicate significant differences ($p < .05$, Kruskal–Wallis and Mann–Whitney tests). Error bars equal to 1 SD.

swimming activity of juveniles (Kihara et al., 2002; Printzi, Fragkoulis, et al., 2021; Sfakianakis et al., 2006). Mazurais et al. (2009) showed that the development of haemal lordosis in European seabass juveniles can be significantly affected by the nutritional conditions (dietary levels of vitamin A) which were applied during the earlier larval period. In the present study, the development of haemal lordosis by zebrafish juveniles during 4 days of SCT, was significantly affected whatever the dry feed provided. This result suggests that appropriate dry feeds could in future be used for the control of lordosis development in aquaculture fish, during the critical early juvenile period. In the present study, 48%–67% of the lordotic fish presented scoliosis of the caudal peduncle. In contrary to haemal lordosis that developed primarily during SCT, caudal-peduncle scoliosis developed primarily before SCT. Both abnormalities however, presented the same response pattern to the tested diets (comparatively decreased in the A dietary group). These results might suggest that both abnormalities have a common mechanism of induction, involving altered musculoskeletal development due to nutritional imbalances. The hypothesis that lordosis induction during SCT is favoured in fish with caudal-peduncle scoliosis might be excluded in the present paper, because only normal fish were exposed to SCT (scoliotic individuals were observed from the top and excluded).

Total diet performance revealed a similar response pattern of A, D and Ctrl fish groups, at the end of the larval rearing. Availability of nutrients (e.g., lipids, proteins, vitamins) during larval development has been proven critical in an ontogenetic and species-specific manner (Boglione et al., 2013). Simultaneously, skeletogenesis has been correlated with the availability of several essential nutrients, such as trace elements, phospholipids and vitamins (Cahu, Zambonino Infante, & Takeuchi, 2003; Darias et al., 2011; Lall & Kaushik, 2021). Indeed, increased early phospholipid provision resulted in earlier ossification of the vertebral column in Atlantic cod larvae (Kjørsvik et al., 2009) and/or decreased skeletal deformities rates in both marine (Cahu et al., 2009; Villeneuve et al., 2005) and freshwater species (Geurden et al., 1998). Remarkably, diets Ctrl, A, D of the present study with the lower abnormalities rates at the end of the larval stage, presented the higher phospholipid contents. Fatty acids and especially EPA and DHA, often included within the PLs, have been reported as crucial for early skeletal formation regulating the expression of key gene families such as BMP and IGF genes by acting on several nuclear receptors (PPAR, RAR and RXR; Izquierdo et al., 2010; Villeneuve et al., 2005). Increased resistance against swimming-induced lordosis could be potentially explained by an earlier vertebral column formation or mineralization. Concerning the variance in protein content among the dietary groups, the hydrolysate level (amino acids, peptides) would be necessary to further determine the potential effects on the skeletal structures (Zambonino Infante et al., 1997). Interestingly, experimental diet Ctrl originally designed for marine species mimicked the zebrafish-specific diets A and D. This observation could be attributed to the similar larval nutrient requirements during the early ontogenetic stages of different species. In the present study, differences in abnormality rates

among the different groups might also be attributed to other feed characteristics, such as the suitability of diet characteristics for zebrafish larvae (e.g., crumble diameter, colour, palatability, digestibility) or for use in freshwater (e.g., sinking rate, leaching rate).

To conclude, in the present, we showed that starter diets significantly affected the skeletal development of zebrafish larvae, with clear effects on the frequency of gill-cover abnormalities and caudal-peduncle scoliosis. Additionally, our results showed that the negative effects of intense swimming on zebrafish vertebral column (in the form of haemal lordosis) were significantly influenced by the provided dry feed. The present study provides a strategy that combines swimming tests with nutritional tests, in order to better identify critical nutrients for bone development (e.g., vitamins, minerals, fatty acids, amino acids), which will lead to a better definition of the nutritional need of developing zebrafish.

AUTHOR CONTRIBUTION

A.A. carried out the experiments and analysed the data; A.A., Ch.K. and S.F. maintained the fish populations. A.A. and G.K. analysed the data; A.P., D.M. and J.Z-I., supervised diet analysis; A.P., Ch.K., and G.K. prepared the manuscript; G.K. designed the research. All authors reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data, including the names of the commercial feeds, can be made available upon reasonable request to the authors.

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