THE ROLE OF STARTER DIETS ON THE DEVELOPMENT OF SKELETAL ABNORMALITIES IN ZEBRAFISH

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Introduction

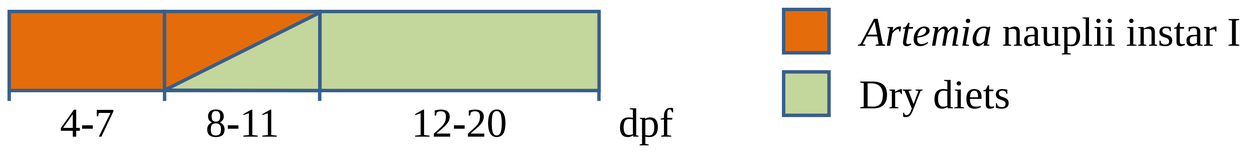
Skeletal abnormalities are a major problem for both aquaculture and laboratory fish with significant impact on the production cost and fish welfare (Boglione *et al.* 2013; Printzi *et al.* 2021b). They mainly develop during larval rearing and early juvenile period, with various factors to be responsible for their development, including adverse abiotic conditions, genetic factors and nutrition (Boglione *et al.* 2013).

In this study, we examined the effect of different starter diets on the development of skeletal abnormalities and survival rate in zebrafish *Danio rerio*.

Materials and Methods

Five starter diets (four commercial, A-D, and one experimental, CTRL) were tested, in triplicate trials (28 °C):

Artemia to dry-feed transition scheme applied



At 18-20 dpf, random samples of 50 individuals were fixed and stained for the examination of skeletal abnormalities. Additionally, from three commercial diets (A,C,D) twenty fish without any gross external morphology (11-12 mm total length, TL) were subjected to swimming-challenge-test (SCT) for lordosis induction (8 TL·s^{-1}, Printzi *et al.* 2021a).

All diets were analyzed with respect to their proximate composition (Table 1).

Table 1. Proximate composition of the tested diets.

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

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

Swimming activity induced haemal lordosis in all the experimental groups subjected to SCT (groups A, C, D). Analysis showed that lordosis frequency in diet A (53.3±17.6%) was significantly lower than in diets C and D (80.0±5.0% and 70.0±5.0% respectively, G-test, $p<0.05$, Fig. 2A, 2D).

Similarly, starter diets significantly affected the development of caudal scoliosis during the swimming-challenge-test (G-test, $p<0.05$, Fig. 2B). Scoliosis frequency increased from 30.0±10.0% in diet A, to 53.0±11.8% and 71.0±6.5% in diet D and C respectively.

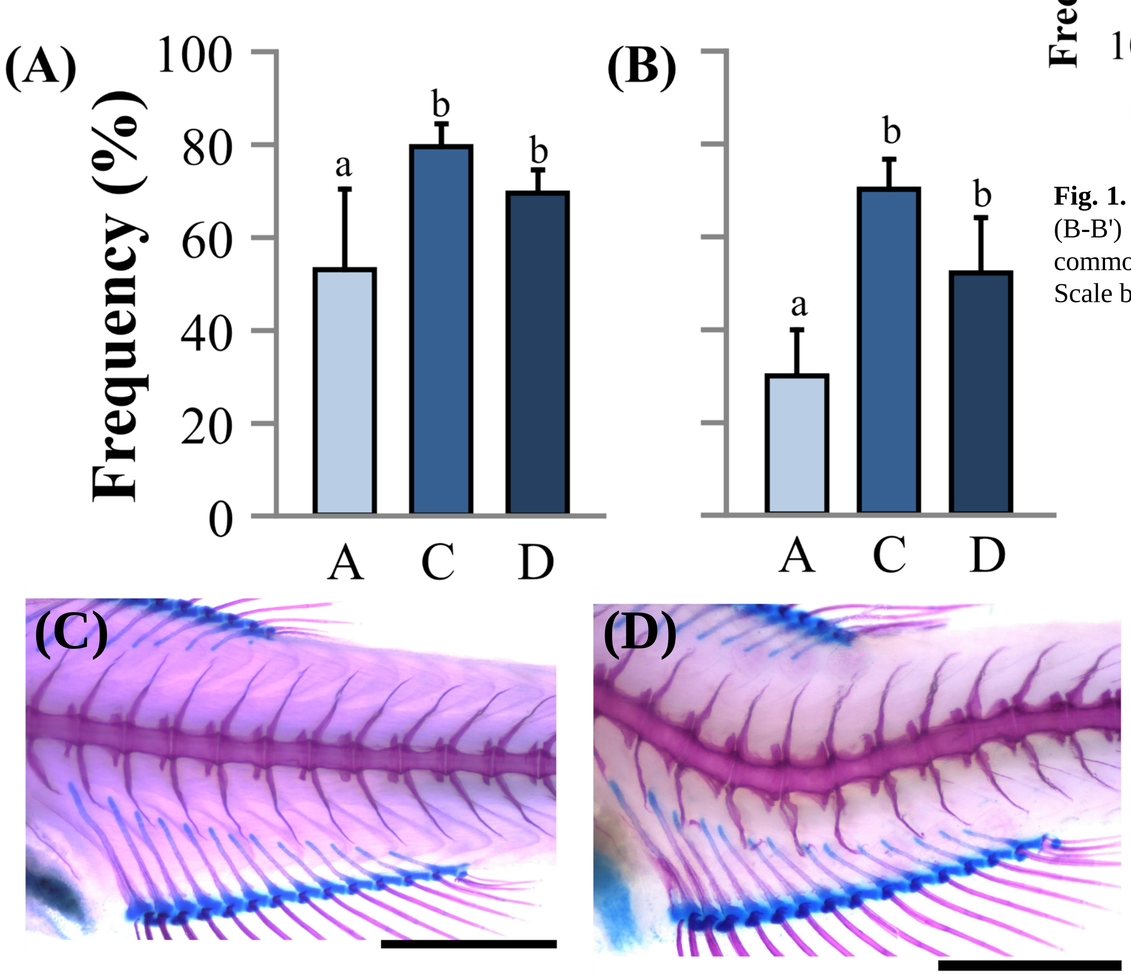


Fig. 2. Lordosis (A) and caudal scoliosis frequency (A, B) in the three dietary regimes, after the SCPT. Representative cases of (C) normal and (D) abnormal haemal vertebrae. (D-D') severe gill-cover abnormalities. The absence of a common letter indicates statistically significant differences between diets (G-test, $p<0.05$). Error bars are equal to 1SD. Scale bars = 1.0 mm.

Results

Starter diets significantly affected the development of caudal scoliosis ($p<0.05$, 19.3-54.7%, Fig. 1B, 1B') and gill-cover abnormalities of both light ($p<0.05$, 11.3-36.7%, Fig. 1C, 1C') and severe ($p<0.05$, 1.3-11.3%, Fig. 1D, 1D') intensity. Similarly, the survival rate was affected by the nutritional regimes that were applied ($p<0.05$, 63.1-90.3%, Fig. 1A).

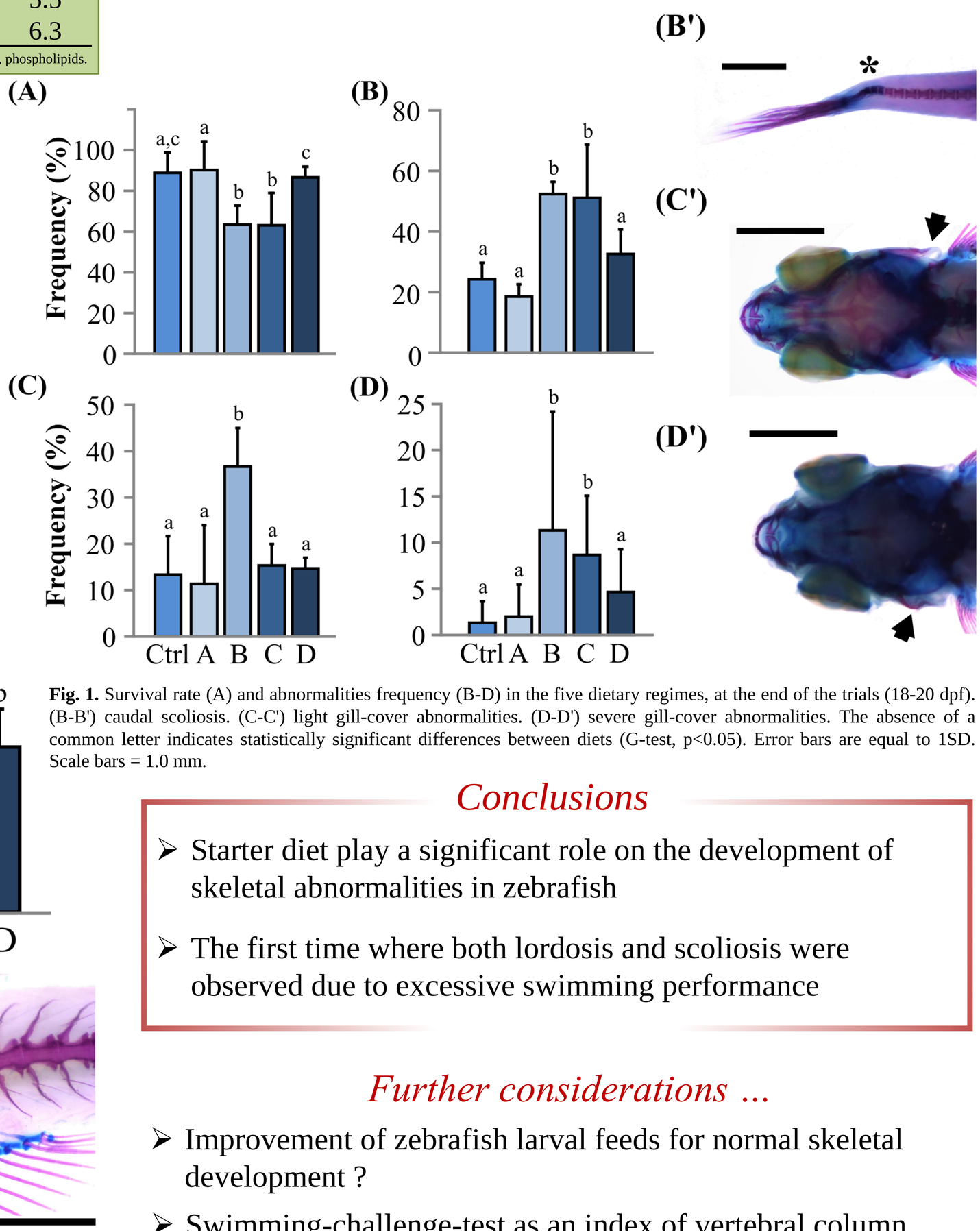


Fig. 1. Survival rate (A) and abnormalities frequency (B-D) in the five dietary regimes, at the end of the trials (18-20 dpf). (B-B') caudal scoliosis. (C-C') light gill-cover abnormalities. (D-D') severe gill-cover abnormalities. The absence of a common letter indicates statistically significant differences between diets (G-test, $p<0.05$). Error bars are equal to 1SD. Scale bars = 1.0 mm.

Conclusions

- Starter diet play a significant role on the development of skeletal abnormalities in zebrafish
- The first time where both lordosis and scoliosis were observed due to excessive swimming performance

Further considerations …

- Improvement of zebrafish larval feeds for normal skeletal development ?
- Swimming-challenge-test as an index of vertebral column integrity in aquaculture species ?

Acknowledgements

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