The effect of dietary phosphatidylcholine/phasphatidylinositol ratios on gilthead seabream (Sparus aurata) deformities

E. Sandel[1], O. Nixon[1], S. Lutzky[1], B. Ginzbourg[1], A. Tandler[1], Z. Uni[2] and W. Koven[1]

[1]Israel Oceanographic and Limnological Research Institute, The National Center for Mariculture P.O. Box 1212, Eilat 88112, Israel (Eran.sandel@huji.ac.il)

[2]Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel P.O. Box 12, Rehovot, 76100, Israel

Introduction

One of the major problems afflicting mariculture today is the high incidence (>30%) of skeletal and morphological deformities. This problem drastically affects the market value of the fish harvest. Previous research hypothesized that dietary phosphatidylinostol (PI) was more effective in reducing deformities than the main membrane phospholipid phosphatidylcholine (PC).

The Aims of the research were:

To determine, through the use of a soft gelatin micro-encapsulated diet, the effect of dietary PC/PI ratio on the incidence of skeletal deformities, osteocalcin (bone Gla protein, BGP) mRNA expression and the performance of gilthead sea bream larvae, juveniles and fry.

Materials and Methods

- Four microdiets (MDs), differing only in their PC/PI ratios and replacing 75% of the normal *Artemia* ration (mg DW), were fed to 20-34 days post hatching (dph) larvae (Table 1). The highest PC/PI ratio diet (lowest PI) was considered as the control (Diet A) while a commercial reference treatment (100% *Artemia* ration) was also tested (Art treatment).
- At 40dph the larvae were graded into small (<1.3 mg larva^{-1}) and large (>2.9 mg larva^{-1}) sizes from each tank and were reared in aquariums (till 67dph) and fry rearing cages (till 141dph).
- Determination of deformities: 67dph juveniles were stained for bone and cartilage using a modified method by Potthoff T. (1984). 141dph fry were manually sorted for phenotypic cranial (jaw) and gill cover deformities. Then, the fry were analyzed using X-ray for skeletal deformities (Plate 1).
- The BGP mRNA levels were tested by RT-PCR from the experimented juveniles in all treatments from five developmental stages (40-83dph).
- All of the Results are expressed as mean ± S.E.M (n=4). Levels within a size group having different letters were significantly (P<0.05) different. Levels between size groups fed the same treatments and having different (*) were significantly (P<0.05) different.

Table 1 The dietary PC/PI ratio in the experimental treatments

	A	B	C	D	Art
(g/100 g dry diet)	25% *Artemia* +75% MD	25% *Artemia* +75% MD	25% *Artemia* +75% MD	25% *Artemia* +75% MD	100% *Artemia*
Phosphatidylcholine	5.7	4.54	4.42	3.88	4.42
Phosphatidylinositol	1.86	1.95	2.76	3.04	1.78
PC/PI in total diet	3.07	2.32	1.6	1.28	2.48

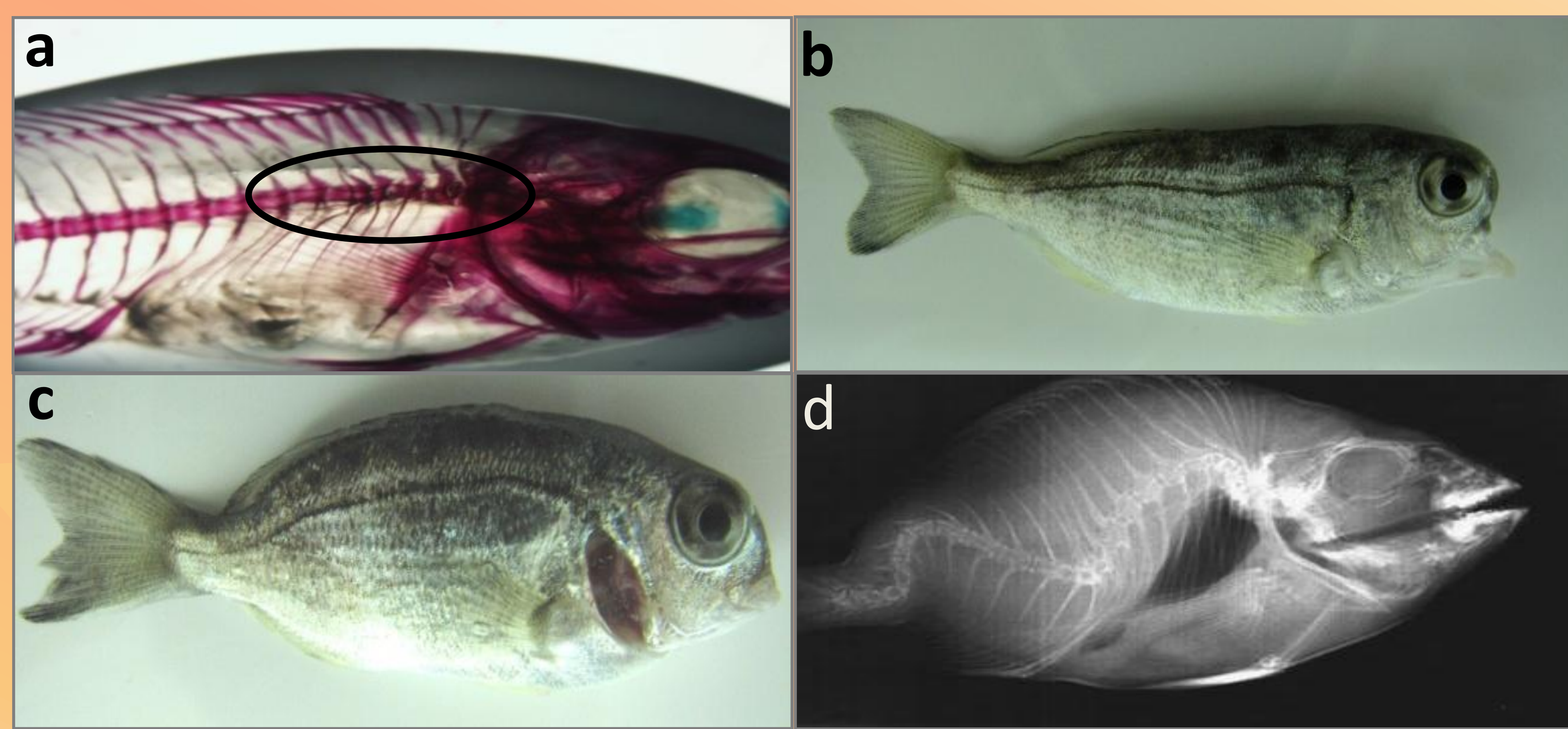


Plate 1 Gilthead sea bream with different malformations: (a) Stained specimen with vertebral compression. (b) Specimen with cranial deformity. (c) Specimen with gill cover deformity. (d) X-ray picture of specimen with Lordosis and Scoliosis.

Results

1. There was no marked treatment effect on growth rate in 40dph larvae. On the other hand in later juvenile development (67dph), decreasing dietary PC/PI ratio contributed to significantly better growth (Fig 1a) and higher survival (Fig. 1b).

2. Decreasing dietary PC/PI ratio significantly reduced cranial deformities (Fig 2a) while induced skeletal deformities (Fig 2b). The skeletal deformity (%) was more marked in faster growing individuals compared to their slower growing cohorts.

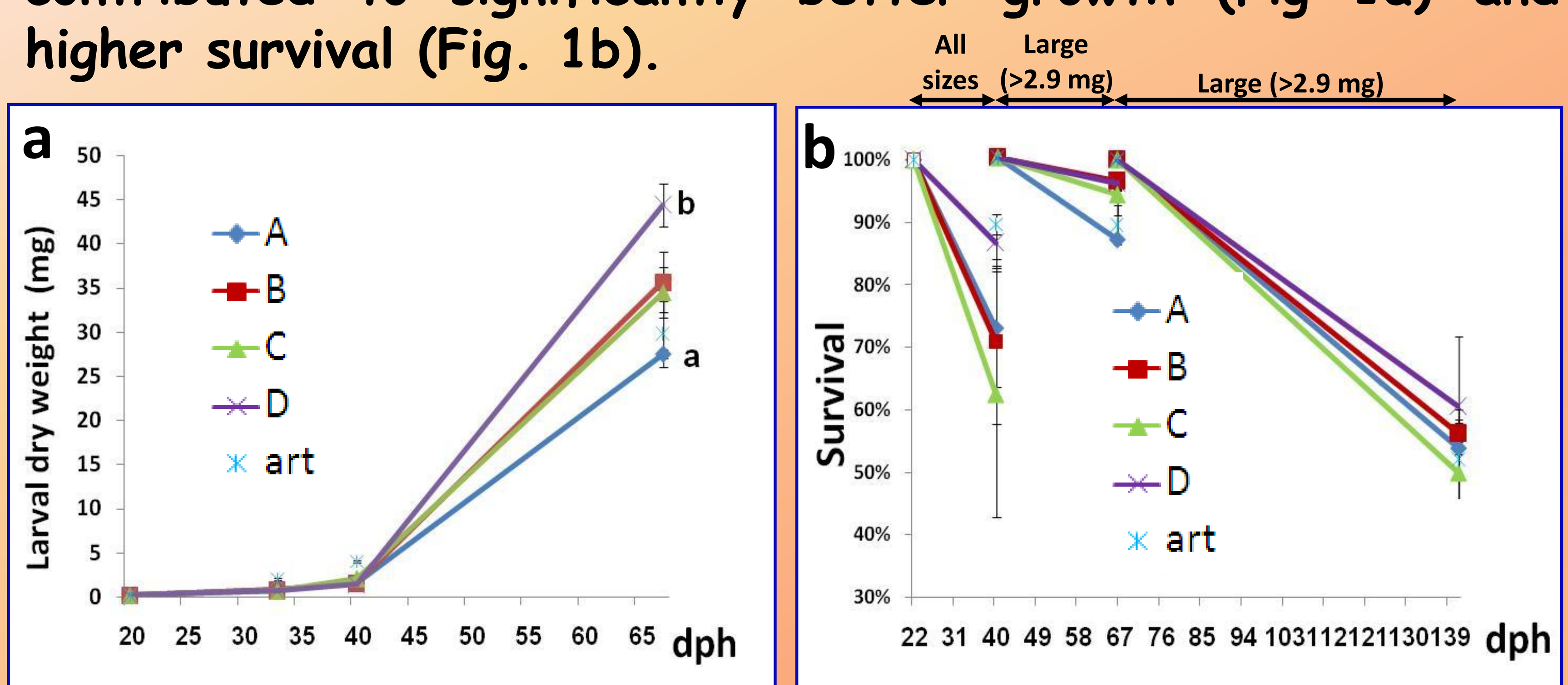


Fig. 1 The effect of dietary PC/PI ratio on large (>2.9mg dw larva^{-1}) larvae (a) dry weight (20-67dph) and (b) survival (22-141dph).

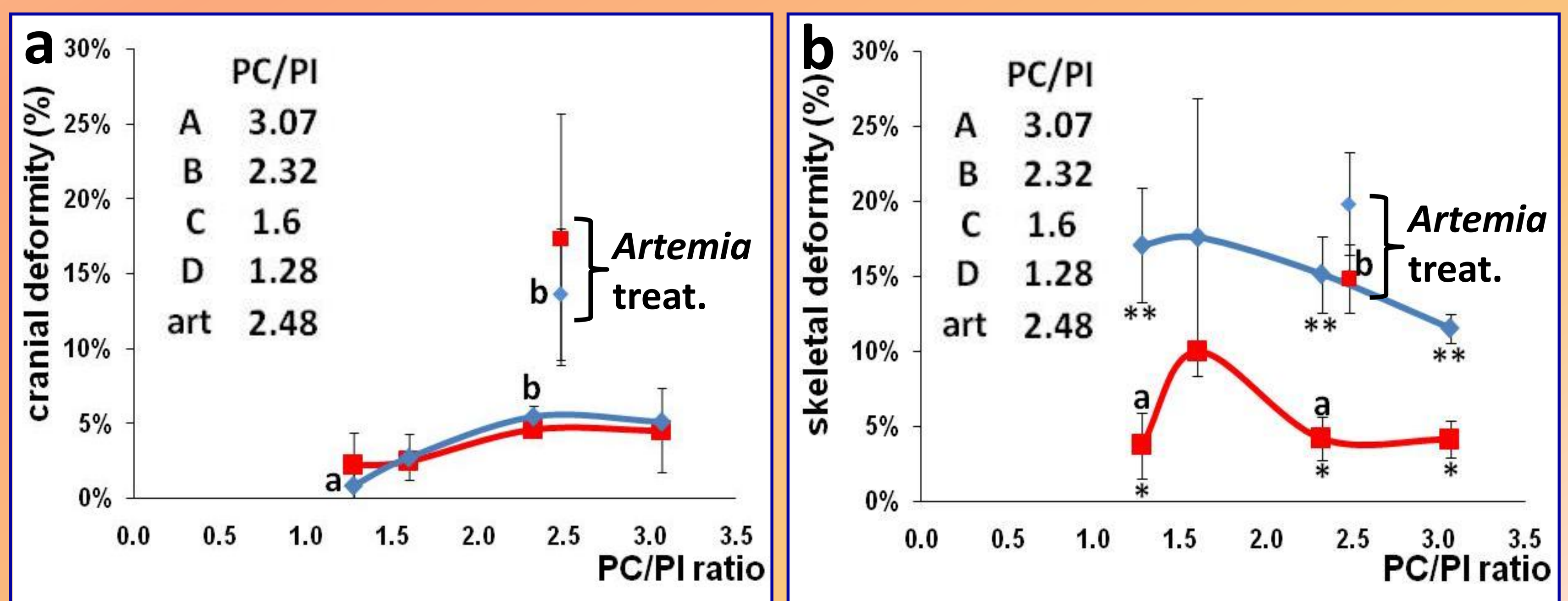
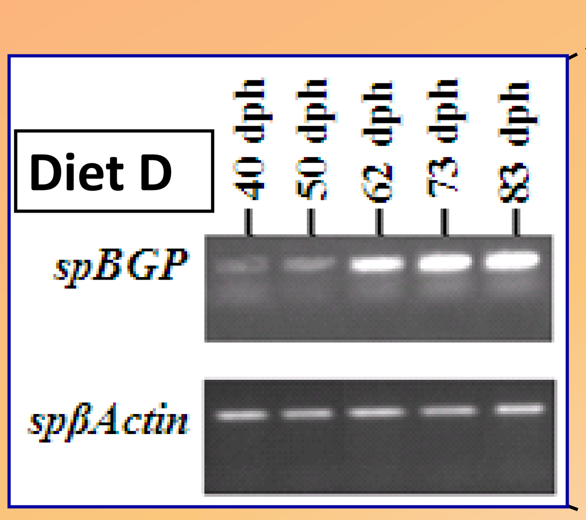


Fig. 2 The effect of dietary PC/PI ratio on (a) cranial and (b) skeletal deformities level (%) in small (<1.3mg dw larva^{-1} –■–) and large (>2.9mg dw larva^{-1} –◆–) juveniles at 67dph.

3. *Sparus* BGP mRNA levels correlated well (R^2= 0.964) with development in the faster growing fish fed the high PI diet (lowest PC/PI ratio) (Fig. 3, 4).

Fig. 3 *Sparus* BGP mRNA by RT-PCR using *spBGP* and *spβActin* specific primers (40-83dph) in large group juveniles fed Diet D.



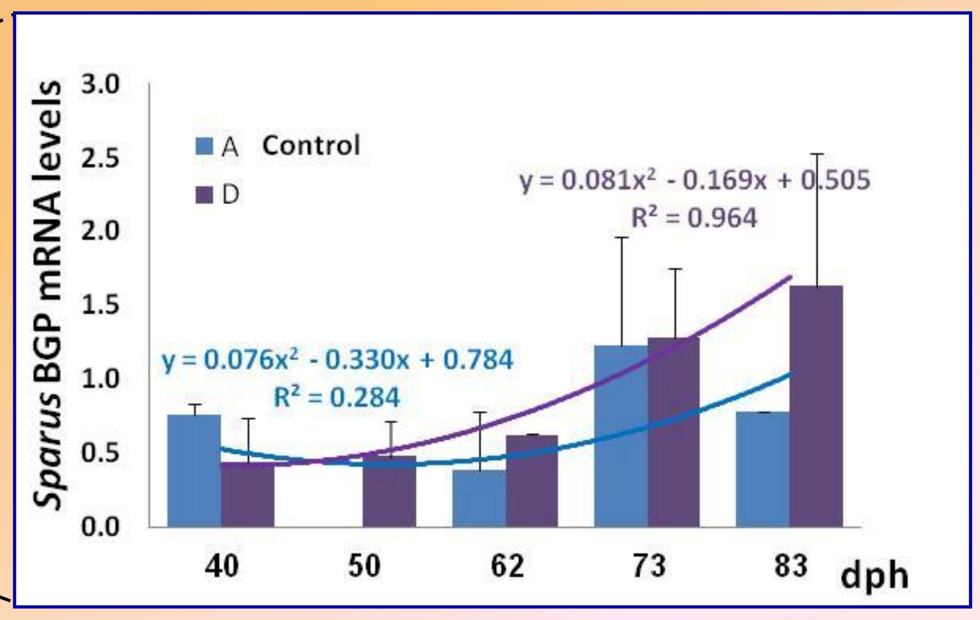


Fig. 4 *Sparus* BGP mRNA levels (40-83dph) in large (>2.9mg dw larva^{-1}) juveniles fed Diet A and D.

Conclusions & discussion

- Reducing dietary PC/PI ratio markedly decreased cranial deformity at 67dph. This may have contributed to improved feeding efficiency on pellets in a later stage leading to significantly (P<0.05) better growth and (P>0.05) higher survival.
- The results suggest that PI is involved in the regulation of osteocalcin (BGP) synthesis which reduced cranial deformity while tending to cause over-mineralization and skeleton deformities in faster growing larvae.
- The most effective dietary PC/PI ratio for sea bream larval and fry rearing was 1.28 (PI level of 3.04g 100g^{-1} diet).

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