

Relationships between absence of functional swim-bladder, calculosis and larval mortality in hatchery-reared gilthead sea bream, *Sparus aurata* L.

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Abstract. The relationship between absence of functional swim-bladder, calculosis and larval mortality of hatchery-reared sea bream, *Sparus aurata* L., was observed. There was a temporal variation in the main causes of mortality, i.e. not inflated swim-bladder appears to be the main cause up to the 47th day, and the presence of calculi around the 55th day. Histological sections, semi-quantitative analysis and scanner electron microscope photographs were carried out.

Introduction

One of the main problems in rearing many different fish species is the high larval mortality rate. This problem cannot be solved only by increasing the broodstock. Among the various causes of larval mortality, the non-activation of the swim-bladder in early development stages (referred to in this paper as no swim-bladder), probably caused by the inability to gulp air because of an oily film on the water surface (Chatain & Dewavrin 1989; Chatain & Ounais-Gushermann 1990), plays a major role. The percentage of *Sparus aurata* L. larvae in culture, showing a correct inflation of swim-bladder can, for example, vary between 0 and 25% (Chatain & Ounais-Gushermann 1990).

Another pathological condition that may influence larval mortality is the frequent presence of calculi in the kidney and near the urinary bladder (Ueda, Ishioka, Okamoto & Fukuara 1970; Paperna 1978; Yamashita 1981; Berg, Moretti & Fredi 1983). In spite of these observations, quantitative data on this pathology and on the relationships between larval mortality and calculosis have not yet been reported.

Without considering the physiological mechanisms of this pathology on larval mortality, the aim of this paper was to investigate the temporal relationship between the non-activation of the swim-bladder or of calculosis in different developmental stages, on the basis of the presence of these pathologies in dead larvae.

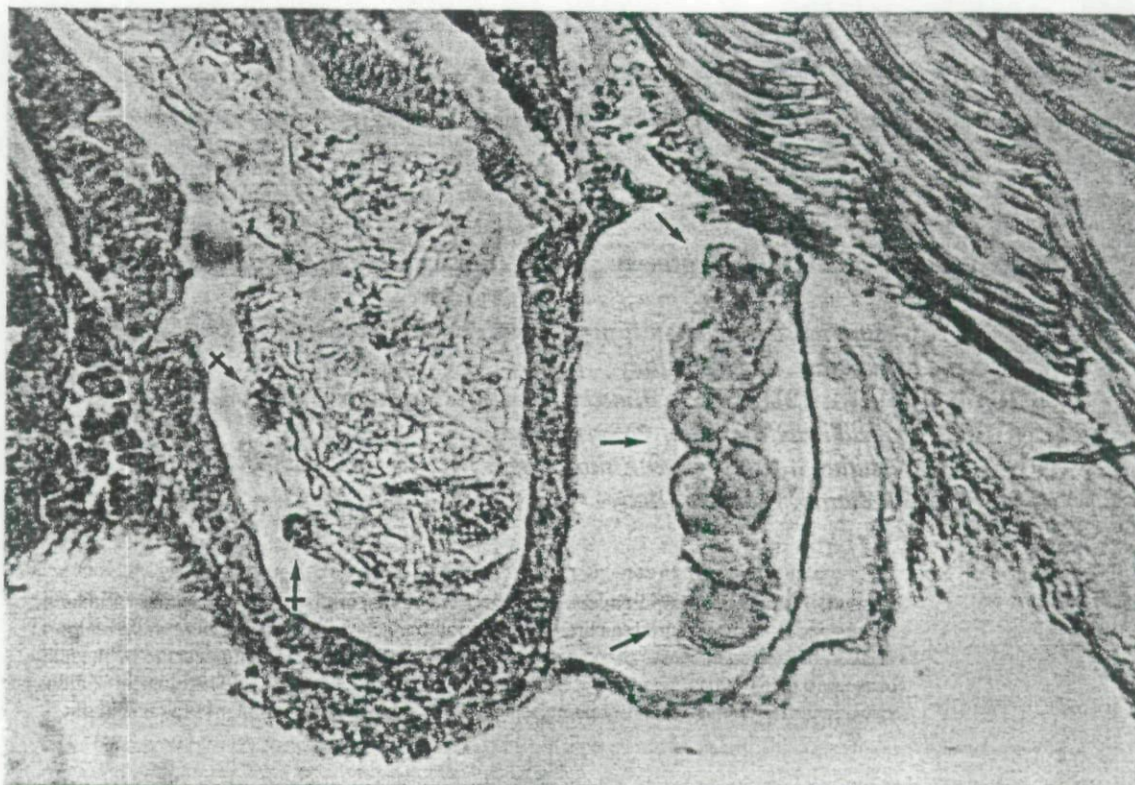


Figure 1. Particular of histological section of *Sparus aurata* larva, stained with haematoxylin and eosin. The arrows show the organic material surrounded by apatite crystals. Crossed arrows indicate the intestinal tract filled with food.

Materials and methods

Sparus aurata larvae, obtained by natural deposition, were reared in conical 2000-l-capacity rearing tanks. To maintain the optimal condition, water temperature ($16^{\circ}\text{C}\pm 1$), pH (8.3) and N-NH_4 ($<0.3\text{ mg/ml}$) were controlled daily.

In the first week the feeding regime was microalgae (*Platimonas suecica*, *Chlorella minutissima* and *Isochrysis galbana*), followed by 3 weeks' distribution of rotifer *Brachionus plicatilis* and 3 weeks of live *Artemia* nauplii (instar I) and enriched nauplii (instar II), until the end of the experiment.

The presence of functional swim-bladder and the presence of calculi were screened in 500 live and 1500 dead animals from the 7th to the 65th day after hatching, by a stereomicroscope.

Specimens were collected randomly with a pipette from a big group of larvae located in the centre of tank, a few minutes after food distribution, whereas all the dead animals were collected daily.

Larvae with swim-bladder and without calculi were considered 'normal'. The relative percentage of abnormal and normal larvae were determined on dead animals.

The semi-quantitative analysis of calculi composition was carried out with EDS (energy dispersion spectrophotometry) microprobe. The Philips 515 scanning electromicroscope was

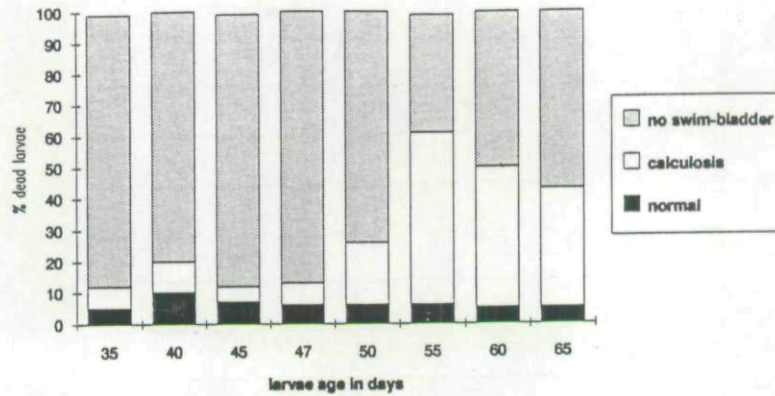


Figure 2. Percentage of calculus incidence compared with normal and non-functional swim-bladder fish, calculated on total dead *Sparus aurata* larvae amount from 30th to 65th day.

used. The surface area of calculi (generally shaped like a cylinder) was arbitrarily chosen as an index of their size.

The calcium determination on *Artemia* nauplii either enriched or not, was estimated by NMR analysis on nauplii suspension.

Results

Larvae intestinal inspection showed the presence of food in either fresh specimens or histological sections (Fig. 1).

Figure 2 shows the percentage of dead animals which had calculi or no swim-bladder, compared with the 'normal' fish. The increase in the surface area of calculi during development is reported in Fig. 3.

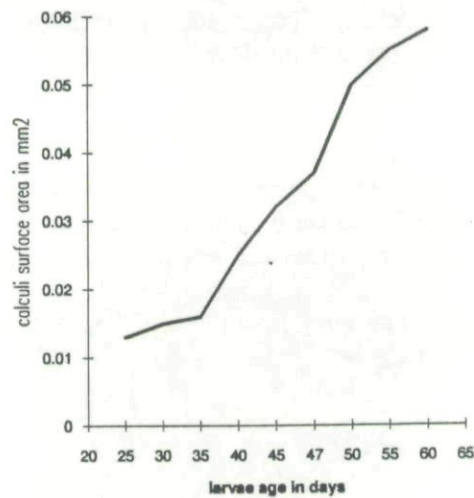


Figure 3. Increase of calculi surface area during observations.

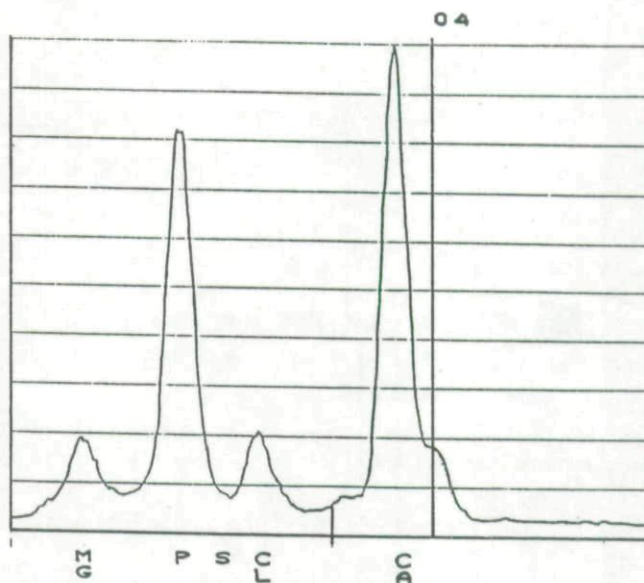


Figure 4. Graph line of semi-quantitative analysis of calculi composition carried out by EDS. Excitation condition: anode: 40 KEV tension (X axis); counter time: 100s (Y axis). Mg = peak of magnesium; P = peak of phosphorus; S = peak of sulphur; Cl = peak of chlorine; CA = peak of calcium.

A peak of mortality was observed in 'normal' animals at 40 days after hatching, during the week when rotifer feeding is substituted for live *Artemia* nauplii. This percentage, however, does not exceed 10%.

Of the dead larvae, 85–90% have no swim-bladder, although this reduced to about 50% after the 50th day (Fig. 2).

The incidence of larvae possessing calculi was about 5–10% in both cases up to day 45 followed in both groups by a successive small drop (Fig. 2). At this time the maximum calculi surface area was 0.025 mm^2 . The maximum incidence of calculi was reached on the 60th day and was about 55% when calculi surface increased to 0.05 mm^2 (Fig. 3).

Histological section of final tract of urinary bladder shows the presence of organized material with a core stainable with eosin (Fig. 1).

A typical spectrum of composition is reported in Fig. 4. Semi-quantitative analysis of the crystal composition, showed high P and Ca percentages, responsible for the formation of apatite crystals $\text{Ca}_5(\text{PO}_4)_2$. This was not pure, and was always accompanied by elements like Mg, Cl and S (Fig. 5).

Ca^{++} concentration in *Artemia* nauplii, grown in enriched medium, was 7 mg/g dw, in non-enriched medium it was 1.2 mg/g dw.

Discussion

Observations can be made on mortality from the analysis of the incidence of normal larvae, functional larvae with no swim-bladder and larvae with calculi.

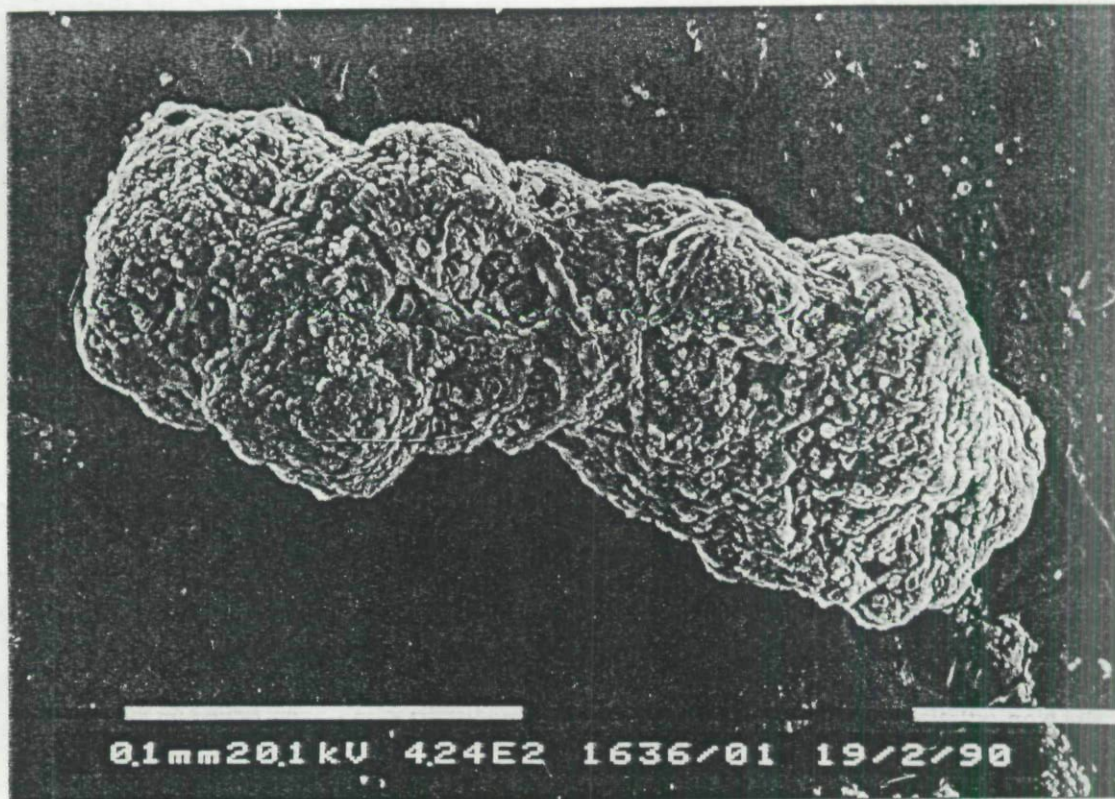


Figure 5. Calculus of *Sparus aurata* isolated by micro-needles and photographed by scanning electromicroscope. NaCl crystals are shaped as regular cubes. Bar represents 0.1 mm.

Because of the fast decomposition of the youngest larvae (7–25th day), significant data on pathology of dead animals start from the 30th day; however, some observations made during this initial period are reported (i.e. calculi size).

Similarity of mortality percentages in normal larvae and larvae with calculi were observed until the 45 days age. This suggests that in this period the influence of small calculi, showing a maximum surface of 0.25 mm^2 , on mortality, is not relevant.

The non-activation of swim-bladder seems to be strictly associated with mortality during the initial period of development. The consequent hypothesis that this phenomenon may be the cause of mortality does not exclude the influence of concomitant morphological or physiological factors not considered in this paper.

When the incidence of dead larvae without functional swim-bladder decreases (at 45 days), that of larvae with calculi increases until it reaches its maximum at day 50, indicating that the two abnormalities are not completely associated but have a different period of influence. However, a synergic effect of the two pathologies cannot be excluded.

The above observations and the contemporaneousness of changes on feeding schedule with larval mortality, suggest the hypothesis that these two parameters, in the first larval period, are in some way linked and associated with the incapacity of *Sparus aurata* larvae to

adapt themselves to a new feeding regime with live *Artemia* nauplii. Swimming ability of larvae without swim-bladder is in fact strongly reduced.

Increase of mortality around the 55th day can be correlated with a change in the *Artemia* stage distribution from instar I to instar II (enriched nauplii) which constitute the only food until the weaning stage (around the 70th day).

The similar trend in mortality of animals affected with calculosis and calculi surface, suggests that calculi have a strong influence on mortality.

We can suggest various hypotheses about the causes of calculi formation:

- (1) state of stress induced by one or more environmental factors (for example, pH variation in water; Ghittino 1985);
- (2) excess of Ca and P in food (for example, in enriched *Artemia* nauplii), or in water;
- (3) endocrine disorder in Ca and P metabolism.

Further studies will be carried out to try to elucidate this problem.

Berg *et al.* (1983) indicated a significant association between the presence of calculi in the excretory apparatus and the absence of food in the gut. These observations are not confirmed by our investigations; in fact all the dead animals, during the whole experiment, always had an intestinal tract filled with food, indicating feeding activity in these larvae.

Our data, based only on the observation of a temporary relationship between functional swim-bladder absence and mortality of larvae with calculi, even if they do not shed new light on the possible mechanism of their action on animal physiology, suggest possible hypotheses on their mechanism and indicate the necessity for further analysis.

Non-inflation of swim-bladder in sea bass and sea bream seems to be solved by the use of surface cleaner (Chatain & Ounais-Gushermann 1990), but even though the physiological process of swim-bladder disorder has not yet been elucidated. Our next research will be devoted to studying the biochemical mechanisms of this phenomenon.

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