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Research Article

Daily Microgrowth Increments in Otoliths of Common Dentex (*Dentex dentex* Linneaus, 1758) Larvae Reared in Culture Conditions

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Abstract: Daily microgrowth was determined in the otoliths of common dentex (*Dentex dentex*, Linneaus, 1758) larvae reared in 24 h illumination culture conditions. Microgrowth in otoliths was measured from the first mouth-opening stage of larvae on day 3 to the end of the larval stage on day 32. The sagitta and lapillus were present and were equal in size in newly hatched larvae, but the sagitta grew faster than the lapillus during further larval development. The first size increments in otoliths were observed at 4.25-4.99 mm total length of larvae (day 11). Structural development of the rostrum edge occurred on the sagitta on day 25. A polynomial relationship was found in daily lapillus growth and daily sagitta growth. This relationship was more pronounced daily lapillus growth compared to daily sagitta growth ($P < 0.05$). A strong positive allometry was found between sagitta and lapillus growth ($r = 0.89$) and between total length and lapillus growth ($r = 0.98$). Overall, the results obtained until the metamorphosis stage showed that there is a positive allometric relationship between total length and daily growth increments in otoliths of this species.

Key Words: Common dentex, *Dentex dentex*, otolith, daily growth, larvae

Kültür Koşullarında Yetiştirilen Sinagrit (*Dentex dentex* Linneaus, 1758) Larvalarının Günlük Otolit Gelişimi

Özet: Sinagrit (*Dentex dentex* Linneaus, 1758) larvalarının 24 saat aydınlatma uygulanan yetiştiricilik koşullarında günlük otolit gelişimleri saptanmıştır. Otolit gelişimleri ağzın açıldığı 3. günden, larval dönemin sona erdiği 32. güne kadar ölçülmüştür. Sinagrit larvalarında, ilk otolit gelişimi yumurtadan çıktıktan sonraki 11. günde 4,25-4,99 mm total boy aralığında tespit edilmiştir. Yumurtadan yeni çıkmış sinagrit larvalarında sagitta ve lapillus eşit oranda gözlenmiş olup, ilerleyen dönemde sagitta, lapillustan daha hızlı gelişim göstermiştir. 25. günde sagitta üzerindeki rostrum kenarında yapı gelişimi görülmüştür. Günlük lapillus ve sagitta büyümeleri polinomial ilişki tespit edilmiştir. Lapilluslardaki günlük polinomial ilişkinin sagittadaki gelişimden daha hızlı olduğu bulunmuştur ($P < 0,05$). Sagitta ile lapillus arasındaki güçlü pozitif ilişki ($r = 0,89$), larvanın total boyu ve lapillus arasındaki ilişki ile benzerlik göstermiştir ($r = 0,98$). Metamorfoza kadar 24 saat ışık koşulları altında elde edilen verilerde sinagrit larvalarında total boy gelişimi ve günlük büyüme arasında pozitif allometri tanımlanmıştır.

Anahtar Sözcükler: Sinagrit, *Dentex dentex*, otolit, günlük büyüme, larva

Introduction

The common dentex (*Dentex dentex* Linneaus, 1758) is a highly valued Sparidae that inhabits the Mediterranean, the Atlantic from the Bay of Biscay to Madeira and, rarely, the Black Sea (1). There are several papers published on its reproduction and physiology (2,3), larval rearing (3,4), nutrition (5,6), immunology (7), and morphological and osteological ontogeny (8).

The otoliths of teleost fishes are complex polycrystalline bodies, which serve as statoliths in the inner ear (9). Otoliths are formed by the accretion of alternating layers of calcium carbonate and protein matrix around nuclei that appear as the precursors of otoliths during embryogenesis (10). One of the largest 3 otoliths (sagitta, lapillus, and asteriscus) is the sagitta and it grows in daily increments that are commonly used to estimate the age and growth of fish. Daily microincrements have been observed in larvae of various fish under optimum rearing conditions. Under less favourable and physiologically stressful conditions,

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microincrement deposition may occur at a rate of more than or less than one per day (11). The most significant and recent techniques to determine the age of fish larvae are otolith microstructure development in larval fish studies. It is well known that deposition of daily microgrowth increments in fish otoliths is a widespread phenomenon, occurring in species of many biotopes and latitudes (12). Factors influencing the growth of fish larvae and thus otolith deposition include photoperiod, temperature, food availability, salinity, and oxygen concentration (13). Of these factors, temperature and food availability are probably the most important (14).

Otoliths provide the most accurate age determination in fish larvae. They are the first calcified tissues in developing fish embryos (15). However, the formation of otoliths differs depending on biological characteristics of species (16). Daily microgrowth increments in marine fish larvae under different culture conditions have been reported in the European sea bass (*Dicentrarchus labrax*) (17), gilthead sea bream (*Sparus aurata*) (18), striped bass (*Morone saxatilis*), red seabream (*Pagrus major*) (19), common sole (*Solea solea*) (20), bluefin tuna (*Thunnus thynnus*) (21), turbot (*Scophthalmus maximus*) and herring (*Clupea harengus*) (22), and striped mullet (*Mugil cephalus*) (15). However, there is a lack of information about annulus formation on scales and otoliths in cultured common dentex. Annulus formation was described only in adult common dentex under cultured and wild conditions (23). The aim of this study was to determine daily microgrowth increments in otoliths of common dentex larvae under culture conditions.

Materials and Methods

Broodstock and egg incubation

Common dentex broodstock, 8 females (2.4 kg mean weight) and 8 males (1.3 kg mean weight), were selected from wild breeders and stocked in an 8 m^3 tank with a seawater supply of 35 $l.min^{-1}$. Frozen cuttlefish (*Sepia officinalis*) and leander squilla (*Palaemon elegans*) were provided daily in excess as primary food sources. The broodstock were subjected to the natural photoperiod (16 h light; 8 h dark) and the water temperatures varied throughout the experimental period between 17.5 and 18 °C. Eggs spawned by the fish group were immediately collected in a recuparator. Following the fertilisation, the viable buoyant eggs were separated from the dead sinking eggs. The eggs were incubated in 50^{-l} incubators supplied with a gentle flow of seawater at 17.5 ± 0.2 °C. Oxygen saturation was over 85%, salinity was 37 ppt and pH was around 7.65. Ammonia and nitrite components were always kept below 0.012 $mg.1^{-1}$.

Larval rearing

After hatching, the larvae were stocked at a density of 80 $larvae.l^{-1}$ in a cylindro-conical tank (15 m^3 volume and 1.5 m depth). The colour of the tanks was dark grey. Larval rearing was carried out in a closed sea water system. Water temperature was kept between 19 and 20 °C. During larval culture, oxygen saturation was maintained over 85%, salinity was 37 ppt and pH was around 7.6. Ammonia and nitrite were kept below 0.01 $mg.l^{-1}$. The water flow rate was adjusted to exchange 5% of total volume of tanks per day. Light was supplied by fluorescent tubes, 80-100 lx, at the surface of the tank. Photoperiod was set on a 24 h light cycle throughout the study. Newly hatched larvae were fed rotifers *Brachionus plicatilis* (mainly *Brachionus rotundiformis*) cultured with algae and enriched with DHA Protein Selco (INVE Aquaculture, Belgium) at a density of 10-15 $rotifer.ml^{-1}$ from day 3 when the mouth opens to day 12-14. Green water, composed of *Nannochloropsis* sp., *Chlorella* sp. and *Isochrysis* sp. at a density of 150,000-200,000 algae $cells.ml^{-1}$, was also supplied to the larval tank. From day 9 to day 17, *Artemia* grade AF 480 (INVE, Belgium) at 4-7 $nauplii.ml^{-1}$ and from day 15 until day 32 *Artemia* grade EG (INVE, Belgium) metanauplii, both enriched with the Selco (Artemia Systems SA, Gent, Belgium) were introduced to the larvae.

Sampling and otolith measurement

Daily samples were taken from the medial water column of the larval tank at the same time every day. Ten larvae were taken daily for otolith extraction until 32 days post-hatching. Samples were preserved in 85% ethanol. Total body length was measured under a microscope with an ocular micrometer lens (nearest 0.01 mm) immediately after the sampling. Both sagitta and lapillus otoliths were extracted under a binocular stereomicroscope using fine forceps and needles. Afterwards, larvae were put on a slide and the otolith was removed by needles from the metencephalon side and

glycerine dropped on the otolith before closing with a coverslip and subsequent examination under an optical microscope (magnification range 600-1000X).

Statistical analysis

All samples were analysed for outliers by means of Dixon's test, while homogeneity of variances was induced by means of Box-Cox transformation (20,21,24,25). Both time series were differentiated by the expression: $\Delta l/\Delta t = (l_{t1}-l_{ti-1}) / (t_i- t_{i-1})$, where growth rate is expressed in micron.day^{-1}, and l_{t1} and l_{ti-1} are calculated for sagitta and lapillus diameters and total length at time t_i and t_{i-1}, respectively.

Results

Larvae hatched 59-60 h (between 14:30-15:30 hours) after fertilisation. Mean total length of the larvae at the time of hatching was 2.45 ± 0.24 mm (mean ± SD), ranging from 2.37 to 2.52 mm. The mouth and digestive tract of the larvae were functional on day 3 and the first feeding occurred on the same day. The completion of yolk sac absorption took place on day 4 and the oil globule was completely resorbed on day 8 after hatching. The sagitta and lapillus were present, ring shaped, and were equal in size at hatching. Daily growth increment of the sagitta started to occur on day 4 and it grew faster than lapillus during the following days. On day 25, rostrum edge structural development of the rostrum edge of the sagitta started to form. Growth increments were found to take place daily and the microgrowth increment was deposited on day 11 after hatching. The third otolith asteriscus was not observed. The diameter and growth rate of the sagitta are presented in Figure 1 and statistics for polynomial fits of differentiated mean day and sagitta diameter of common dentex larvae were calculated as f: 8.367; $P < 0.05$.

Daily diameter and growth rate of the lapillus are presented in Figure 2 and statistics for polynomial fits of differentiated mean day and lapillus diameter of common dentex larvae were estimated as f: 815.273; $P < 0.05$.

The relationship found in daily lapillus growth was higher than that found in daily sagitta growth ($P > 0.05$). Although the sagitta-lapillus relationship was linear, there was a residual. After removal of the residual according to Dixon's test, a strong positive allometry ($r = 0.89$) was found between sagitta and lapillus growth (Figure 3A). Cubic polynomial graphics and data obtained by

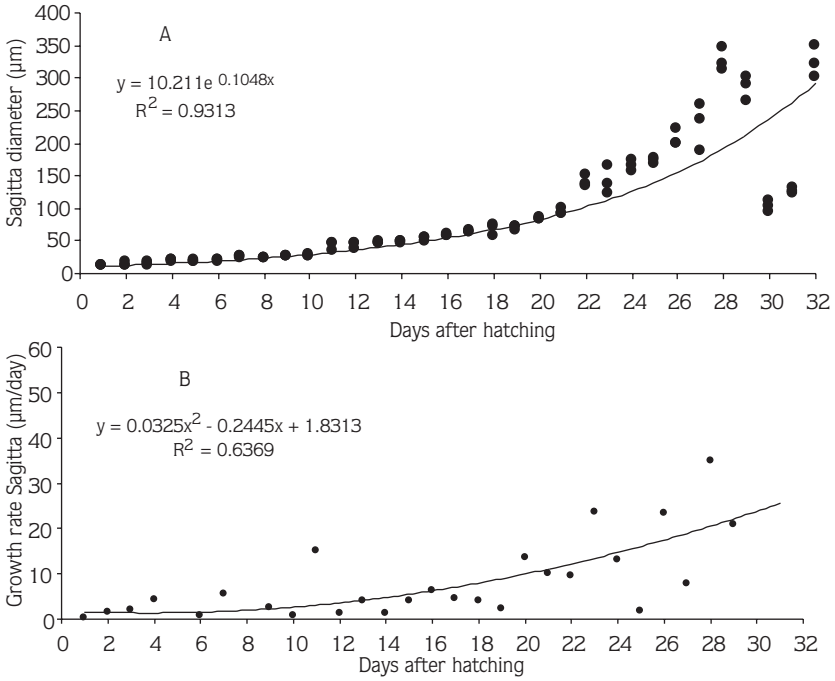

Figure 1. Daily diameter (A) and growth changes of sagitta (B) obtained by differentiating the means of the time series and their polynomial fits.

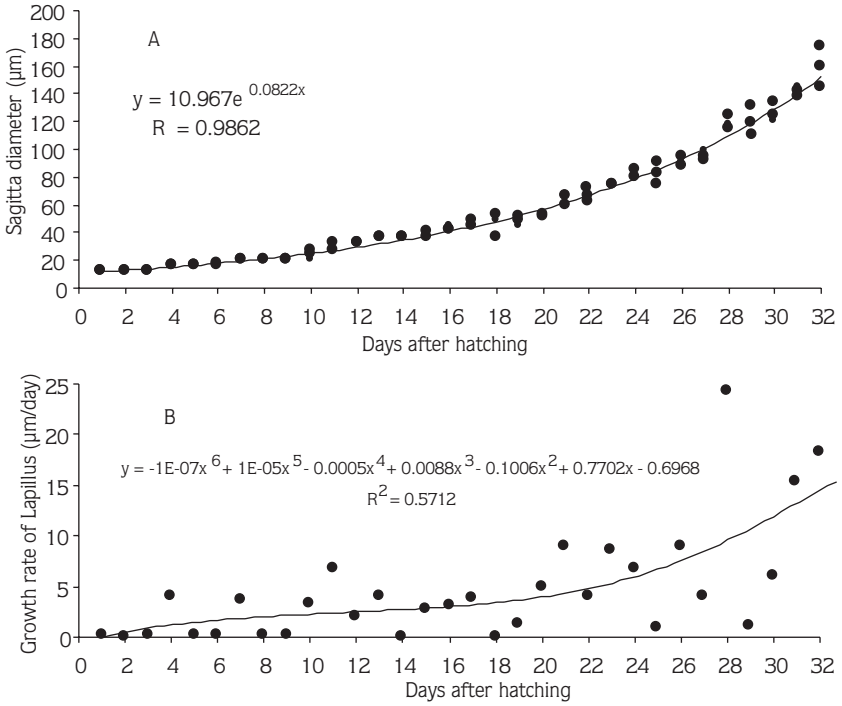

Figure 2. Daily diameter (A) and growth changes of lapillus (B) in *D. dentex* larvae reared in 24 h illuminated culture conditions at 19-20 °C.

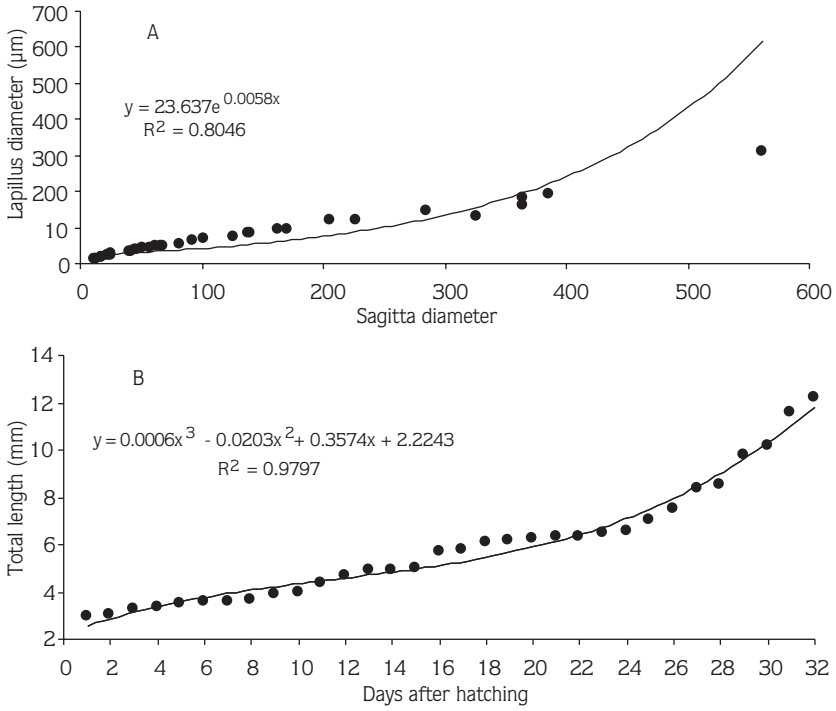

Figure 3. A: The relationship between sagitta and lapillus diameters of *D. dentex* larvae reared in 24 h illuminated culture conditions at 19-20 °C, B: Daily growth rate of lapillus obtained by differentiating the means of the time series and their polynomial fits for *D. dentex* larvae reared in 24 h illuminated culture conditions at 19-20 °C.

logarithmic conversion on Dixon's test due to extreme data on daily total length developments are shown Figure 3B and statistics for polynomial fits of differentiated mean day and total length of common dentex larvae were found as f: 450.424; $P < 0.05$.

Linear regression was used to evaluate the relationships between total length and sagitta, total length and lapillus, and sagitta and lapillus growth. Before using this model, Dixon's test was performed for residuals among the groups.

A strong relationship was found between the total length of the larvae and sagitta diameter ($r = 0.97$). A linear relationship was found between total length of the larvae and lapillus diameter and r was calculated as 0.98 (Figure 4A, B).

Discussion

Daily microgrowth increments of *D. dentex* larvae were investigated in hatchery conditions under constant temperature (19-29 °C) and 24 h illumination. The otolith is one of the most important tools for understanding the life of fish and fish populations. Growth rings (annuli), not unlike those of a tree, record the age and growth of a fish from the date of hatch to the time of death. Daily growth rings formed in the first year of life, and visible only under an optical microscope, record daily age and growth patterns in detail. Furthermore, sophisticated chemical techniques allow the reconstruction of everything from the year of hatch, to migration pathways, to the temperature of the water. Indeed, virtually the entire lifetime of the fish is recorded in the otolith (12,16).

Food availability and temperature are the most important factors influencing larval growth and microincrement of the otolith. The positive relationship between larval growth and otolith microgrowth indicates that quantity (amount) and quality of food (biochemical composition) directly affected these growth parameters. For several fish species, it has been shown that the first daily increment is normally deposited at the end of the yolk-absorption period, when eye pigmentation is complete and jaws are functional (26-28). After completing yolk-sac absorption on day 4 and a few days

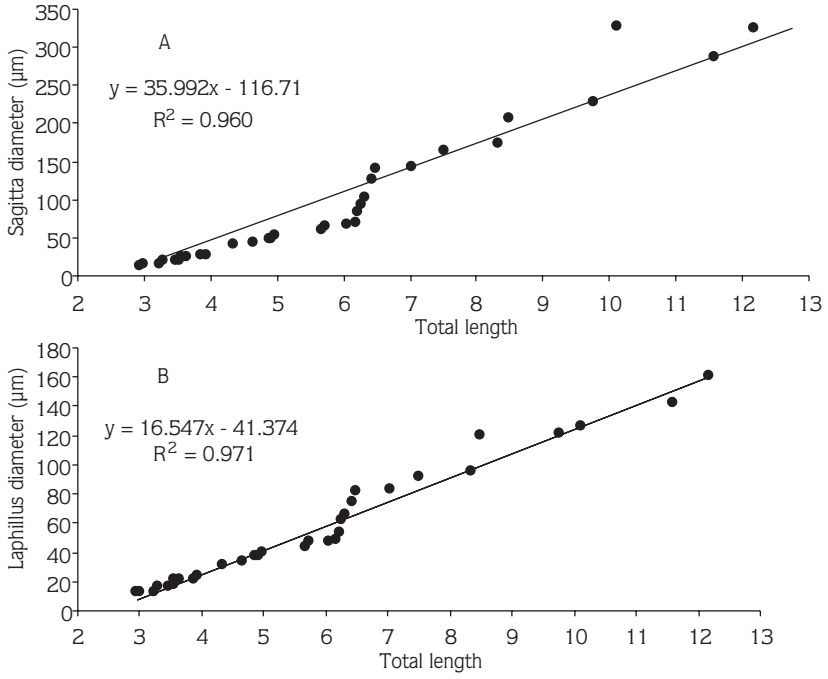

Figure 4. Total length–sagitta diameter and total length–lapillus diameter relationships of *D. dentex* larvae in 24 h illuminated culture conditions.

later, a sharp increase in otolith microgrowth was observed on day 11 when the larvae were preying on *Artemia*, and this result was similar to those found for *D. labrax* (17,24).

Moreover, a strong relationship between food availability and otolith microgrowth was reported for sardine and anchovy caught from their natural habitats (22,29). In sardine, a sharp increase in otolith microgrowth was observed in 14-day-old larvae. At this stage, the larvae were vertically distributed because of the swimbladder inflation. It is commonly known that otolith microgrowth is affected by sudden changes in water temperature (13). In our study, temperature was maintained at 19.6 ± 0.2 °C and hence any negative effect of temperature on microgrowth was prevented.

Another factor influencing daily microgrowth increment is photoperiod. However, a previous study showed that light regime did not affect the number of increments formed per day but it had a marked effect on their sharpness (17,24). This idea was also supported by another study, which was performed on sole larvae (*Solea senegalensis*) (29,30). In our study, we maintained a constant photoperiod level (24 h illumination), which is observed to favour the survival rate of larvae.

Polynomials are rarely used only for evaluation of biological data; they are also used to point out strong relationships after removing the residuals. In the present study, we polynomially described relationships between daily sagitta growth-total length and daily lapillus growth-total length development of the larvae. It could be stated that a new modelling technique was established for day-sagitta relationships due to the relatively low day-lapillus relation. As mentioned above, additionally, biotical and abiotical factors such as feeding regime, temperature, salinity, and light intensity affecting culture conditions could be described. Furthermore, the total length-sagitta relationship r (0.97) was lower than total length-lapillus relationship r (0.98). Data obtained under 24 h light conditions until metamorphosis (day 32) showed a positive allometric relationship between total length development and daily growth increment in *D. dentex* larvae. Company et al. (5) described the asteriscus at the end of the larval stage of sea bream (*Sparus aurata*); however, in our study the asteriscus was not observed during larval stage of *D. dentex*. It was reported that the total length of *D. dentex* larvae until metamorphosis changed from 10 to 24 mm (8). In our study, metamorphic changes in larvae occurred on day 32 when the larvae were 12.17 mm in total length. At the same time, these changes were observed in otolith formation. It is thought that these differences were caused by differences in culture conditions and techniques.

It is not clear that the daily microgrowth increment of larva was affected by regulation systems such as osmoregulation, stress, starvation, and irregular environmental conditions (12,13,17,21). Therefore, otolith microstructure can play a major role for daily recording of both environmental and physiological changes, being more important in aquaculture systems. In this respect, the otolith is one of the most critical objectives to determine daily microgrowth and, as pointed by Re et al. (17), may one day be regarded as the "black box", helping to determine important recent events in the early life history of larvae.

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