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Larvae Development Stages of the European Flat Oyster (*Ostrea edulis*)

Sefa Acarli* and Aynur Lok

Department of Aquaculture, Fisheries Faculty, Ege University, Bornova 35100, Izmir, Turkey

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Key words: flat oyster, *Ostrea edulis*, larvae stages, larvae development, pediveliger

Abstract

This paper reports on the larvae development of the flat oyster, *Ostrea edulis* (Linnaeus, 1758), from veliger to pediveliger stage. Adult oysters were induced to spawn by thermal stimulation and large amounts of veliger larvae were obtained for study. Veliger larvae were cultured in 180-l bins at a density of 3 larvae/ml. Larvae, reared at 20±2°C and fed 8-200 x 10^3 cells/ml of *Isochrysis galbana*, reached pediveliger larvae in 17 days. Shell length and width at the beginning of the pediveliger stage were 254 and 233 μm, respectively. Survival rate from veliger to viable pediveliger stage was 15.5%.

Introduction

Oysters are the most common of all bivalves and have been known to man as edible and delicious for centuries. Two oyster species have been reported along the Turkish coast: *Ostrea edulis* (Fischer et al., 1987) and *Crassostrea gigas* (Dogan et al., 2006). These species are considerable resources for human consumption and obtain high market prices.

Earlier studies on *O. edulis* dealt with growth (Alpbaz et al., 1990; Alpbaz et al., 1993), reproductive biology (Yolkolu and Lok, 2000), and aquaculture (Hindioglu and Alpbaz, 1991; Lok and Acarli, 2006). Larvae development in several bivalve species has been studied, providing basic knowledge for the aquaculture of oysters (Loosanof and Davis, 1963; Loosanof et al., 1966; Utting and Spencer, 1991). Larvae stages in oysters were defined by Waller (1981) and Elston (1999), while shell morphogenesis was studied by Carriker and Palmer (1979).

* Corresponding author. Tel.: +90-232-3884000, ext. 1306, fax: +90-232-3883685,
e-mail: sefa.yolkolu.acarli@ege.edu.tr

The aim of the present study was to describe development stages, growth, and survival rates of *O. edulis* larvae from the veliger to the pediveliger stages.

Materials and Methods

Ripe adult individuals of *O. edulis* were obtained from Mersin Bay in the Aegean Sea, Turkey, in 2003. To induce spawning, oysters were cleaned of encrusting organisms, left out of the water overnight (Satuito et al., 1994), and submitted to a thermal shock of 26°C. Immediately after release from the pallial cavity of the parent oyster, larvae were transferred to three 180-l tanks at a rate of 3 larvae/ml and cultured in 20±2°C sea water, filtered at 1 μm and sterilized by ultra-violet treatment. The larvae were fed 8-200 x 10^3 cells/ml *Isochrysis galbana* obtained from the experimental hatchery of the Fisheries Faculty at Ege University. Microalgae were cultivated in batch cultures at 25-27°C. Only cultures in the log phase of growth were used for feeding. Larvae were fed by the method of Tibile and Singh (2003).

Trials were initiated with normally developed veligers at a density of 3,000 larvae per liter. The larvae cultures were maintained at a salinity of 36-36.2% and 20±2°C, the optimal temperature for larvae development (Davis and Calabrase, 1969; Beiras et al., 1995). The culture media was completely changed every 48.h, when 50 larvae from each tank were sampled to record length (anterior-posterior), width (dorsa-ventral), and morphological changes in the velum and umbo area. Larvae were measured with an ocular micrometer according to Satuito et al. (1994).

The instantaneous growth rate (K) was calculated according to the formula: $K = (\ln L_t - \ln L_0)/t$ (Brown and Robert, 2002), where L_t is the measured length at time t, L_0 is the length at the start of the experiment, and t is the number of days since the start of the experiment. The relationship between shell length and width was described by the linear equation: $L = a + bW$, where L is the shell length, W is the width, and a and b are fitted parameters.

Three 1-ml samples were taken to determine mean survival according to the formula: survival = $(N_t/N_0) \times 100$, where N_t is the number of live larvae at time t and N_0 is the number of live larvae at the beginning of the experiment.

Data were pooled for regression analysis and analyzed with one-way ANOVA to examine the effects of each passing day on growth. Survival data were arcsine transformed before statistical treatment and analyzed by Chi-square (χ^2). Differences were considered significant when $p<0.05$. All statistics were executed using SPSS software.

Results

Four larval stages were observed during the 17-day experiment: veliger, early umbo, umbo, and pediveliger (Fig. 1).

Veliger stage. When newly released from the pallial cavity of the adult oysters, the veliger larvae had a mean size of 147±5.0 x 126±7.2 μm (SE = 3.28, n = 50). The veliger stage is also known as the straight hinge or 'D' shell stage. During this stage, larvae are semi-transparent and the velum protrudes, creating a strong ciliary current. This stage lasted 5 days during which no mortality was observed (Table 1, Fig. 2). When the air flow was closed, veliger larvae swam near the surface of the tanks.

Early umbo (veliconcha). On the fifth day, the first larvae entered the early umbo stage. The shape of the larvae changed and resembled a ball. The umbo became slightly oval. At this stage, larvae were less mobile than in the veliger stage and swam through the water column. Some of the larvae shells were broken. Larvae measured 210±8.99 x 185±12.11 μm.

Umbo stage. At the end of the eighth day, the first larvae entered the fully-developed umbo stage. The umbo area was distinctly observed and larvae averaged 239±30.32 x 208±29.26 μm. Larvae swam more slowly than in earlier stages. The cilary part of the velum was also less active. The majority of larvae reached the umbo stage by day 14.

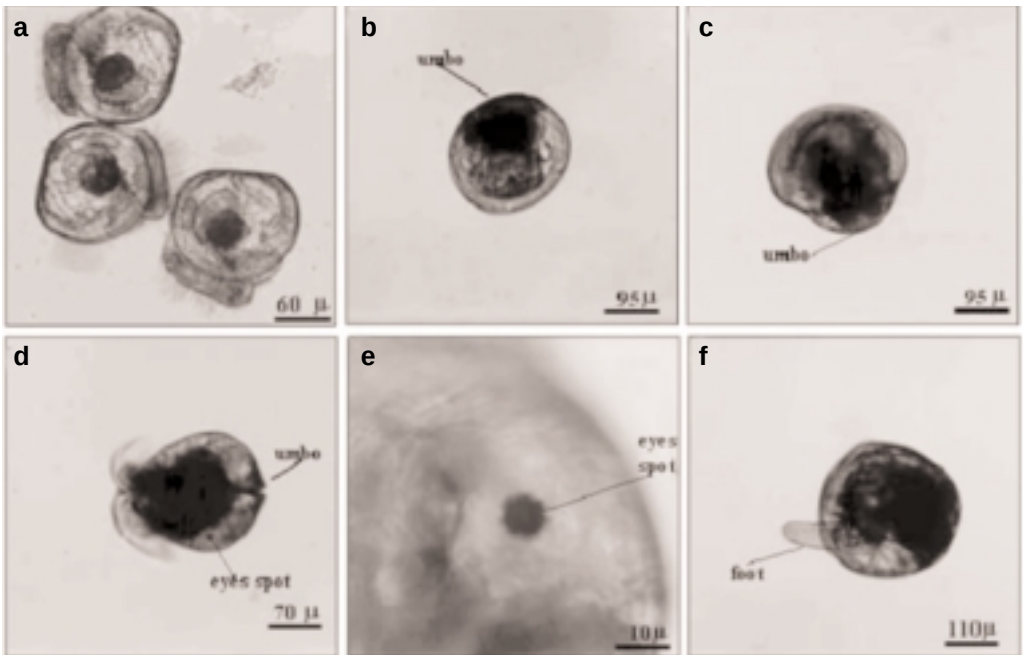

Fig. 1. Larvae stages in the European oyster (*Ostrea edulis*): (a) veliger, (b) early umbo, (c) umbo, and (d-f) pediveliger.

Table 1. Development of *Ostrea edulis* larvae.

	Larvae stage (%)			
Day	*Veliger*	*Early umbo*	*Umbo*	*Pediveliger*
1	100	-	-	-
2	100	-	-	-
5	100	-	-	-
8	28.71	28.71	42.58	-
10	23.81	19.04	57.15	-
12	14.89	14.89	70.22	-
14	9.52	-	90.48	-
17	-	6.6	71.2	22.2

Pediveliger stage. The first larvae reached the pediveliger stage by day 17. A functional foot and eye spot were easily observed in this stage. Larvae congregated deeper in the water column and near substrates, moving along surfaces propelled by their prominent foot and beginning to seek suitable attachment grounds. At the end of the 17-day experiment, larvae ranged 240-300 µm in length and averaged 254±33.37 x 233±33.83 µm.

Growth rates. The average growth rate was 7.87 µm/day from veliger to early umbo stage, 4.57 µm/day from early umbo to umbo stage, and 5 µm/day from umbo to pediveliger stage. The best instantaneous growth rates for shell length and width were observed on days 8 (0.04475) and 10 (0.044228), respectively (Fig. 3). Growth sharply dropped on day 12. Shells had a total increase of 107 µm in both length and width. The average daily growth was 6.29 µm/day. The relationship between shell length (L) and shell width (W) was linear and described

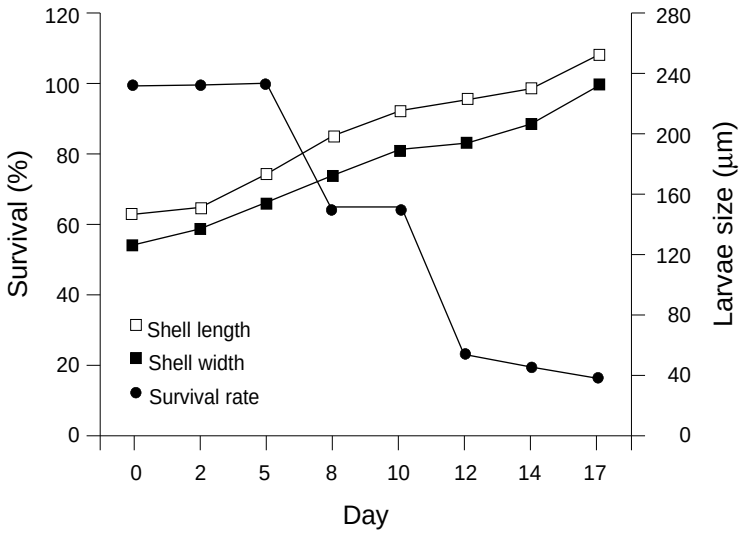

Fig. 2. Survival, shell length, and shell width of *Ostrea edulis* larvae cultured for 17 days from the veliger to the pediveliger stage.

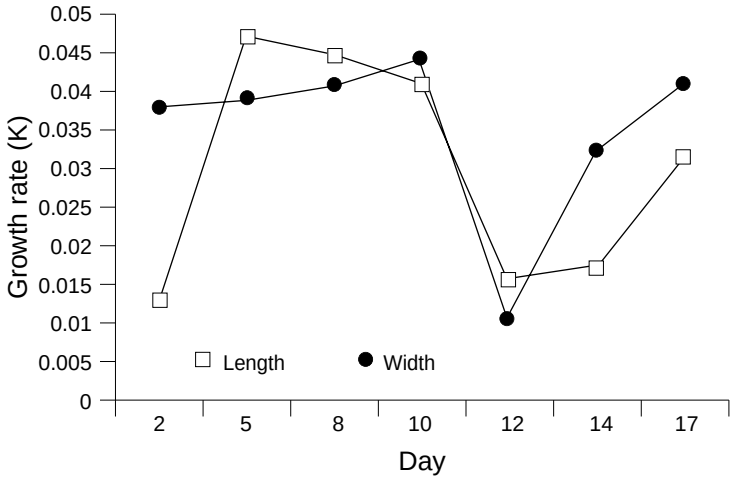

Fig. 3. Instantaneous growth rate of *Ostrea edulis* larvae during the study period.

by the equation L = 1.068W + 13.143, r = 0.9313 (Fig. 4). The effect of day on larvae growth significantly differed between days (p<0.01).

Survival. Survival to the pediveliger stage was 15.5%. Mortality significantly differed from veliger to pediveliger (p<0.05). Mortality was heavy between days 10 and 12.

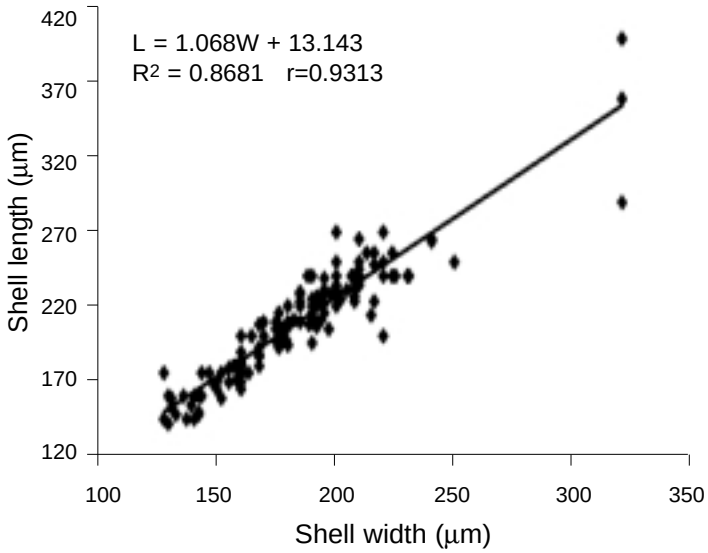

Fig. 4. Relationship between shell length and width of *Ostrea edulis* larvae.

Discussion

Bivalve broodstock can be induced to spawn by thermal or chemical stimulation of the sperm or egg, or electric or mechanic shock (Loosonoff and Davis, 1963). Thermal stimulation is preferred since temperature fluctuation induces spawning with less injury. In the present investigation, *O. edulis* were induced to spawn by fluctuating water temperature from 18°C to 26°C. This method of spawning bivalve species was also reported by Myers and Boisvert (1988).

In general, morphological features of pediveligers, such as a functional foot, presence of an eye-spot, and shell size, are important indicators of competent metamorphosis in bivalve larvae (Utting and Spencer, 1991; Helm and Bourne, 2004). In our study, larvae grew from veliger to pediveliger within 17 days. In contrast, larger pediveliger larvae of *O. edulis* (280-290 µm in length) have been obtained within 9 or 10 days (Walne, 1966). In *Crassostrea gryphoides*, larvae similarly reached the pediveliger stage on day 18 (at 23°C) but at 300-350 µm (Tibile and Singh, 2003) or on day 20 at 280-300 µm (Utting and Spencer, 1991).

Larvae of bivalves have exponential growth (Gosling, 2007) and growth rates are likely to be influenced by genetic factors, endogenous and exogenous nutrition, and culture conditions (Doroudi and Southgate, 2003). The activity of bivalves is generally controlled by water temperature (Tomaru et al., 2002). In *O. edulis*, shell growth is especially affected by water temperature (Wilson, 1987). High temperatures and frost are not tolerated by *O. edulis* (Bardach et al. 1972; Korringa, 1976). Larvae have been successfully reared from veliger to pediveliger at 18-20°C (Russell, 1963) while salinity within the range 25-35‰ has little effect on growth of *O. edulis* larvae (Davis and Ansell, 1962). Because of this, water temperature and salinity were kept at 20±2°C and 36‰ in our study.

Survival was 100% during the first five days and only 15% at the end of the experiment. This relatively poor survival contrasts with the findings of Davis and Calabrese (1969) who reported 70% of cultured *O. edulis* larvae reached pediveliger larvae stage in hatchery trials. Larvae of *O. edulis* are more sensitive than larvae of other oyster species such as *C. gigas* or *C. virginica*.

Therefore the success of culturing *O. edulis* larvae is low. In the present study, survival and the growth rate sharply decreased on day 12. The reason for the decline can be morphological changes in larvae from veliger to umbo stage or unknown changes in the water quality.

In conclusion, larvae developmental stages were defined using standard culture methods. Further research is required to establish feeding standards (algae species and densities) during different larvae stages, determine the effects of salinity and temperature on larvae growth/survival, and examine the biochemical composition of broodstock and larvae.

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