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A Preliminary Study on Reproduction and Larval Development of Swan Mussel [*Anodonta cygnea* (Linnaeus, 1758)] (Bivalvia: Unionidae), in Lake Çıldır (Kars, Turkey)

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Abstract

In this research, reproduction time and larval development of *Anodonta cygnea* in Lake Çıldır during 2000-2002 were studied. The mean number of larvae (N) produced by each gravid female was about 115,000 and the mean shell length was (L) 99 mm (59.2-128.3). Best fitted relationship between the number of larvae and mussel length was found as N = 43.696*$L^{1.68}$ (n = 34, r = 0.42). There was a weak relationship between the length of the mussel and the weight of gills full of larvae, as W = 0.0007 $L^{1.958}$, r = 0.68 (P<0.01). The weakest relationship was found between length (LL) and height (HL) of larvae: HL = 0.2682LL + 256.47 (r = 0.23) (P<0.05). The duration of gravidity was observed from the end of July to June the following year.

Keywords: swan mussel, Lake Çıldır, *Anodonta cygnea*, glochidium, reproduction.

Introduction

Reproduction of the Unionacean freshwater clams is unique in the Unionidae family, in which most species are dioecious. *A. cygnea* populations consist of only a few females and a large proportion of hermaphrodites (Bauer, 2001).

Molluscs set their eggs and sperm free, but the freshwater bivalves of family Unionidae have a more peculiar mode of reproduction. The ripe eggs in a female, pass from the ovaries to the suprabranchial chambers where they are fertilized by sperm discharged to the water column from a male. The eggs become attached by mucus within the water tubes of her gills which enlarge as brood chambers (marsupials). In some species all four gills are used to keep larvae but in some others only the two are used for this purpose. Generally, the life cycle of freshwater Unionid mussels has a parasitical larval stage known as *Glochidium parasiticum* (Jones, 1950).

Wacthler *et al.* (2001) reported that Rathke (1797) had firstly described, along its larval stages, the glochidia as bivalve parasites. Each egg develops to a minute larval glochidium 0.1 to 0.4 mm wide, with two valves closed by an adductor muscle and long larval thread. *A. cygnea* larvae have the valves bearing hooks ventrally. Glochidia are stored within pouches (marsupium) of modified gills. In the formation of the marsupial, one or both pairs of gills may be involved: the outer pair in Unionidae, inner pair in Hyriidae and both pairs in Margaritiferidae. In the spring or summer season, the glochidia are expelled into the water to begin the parasitic phase of their life (Cumming and Mayer, 1992; Wacthler *et al.*, 2001). These are obligate parasites on fish and each species of mussel requires specific host fish in order to complete their development. The larvae are released into the water through the females' exhalent siphon, then sink to the bottom or are scattered by water currents. They can open or close their shells but cannot move independently. Once shed by the female, glochidia must acquire a suitable host or die, usually within 24 hours. Liberated glochidia may travel miles downstream in currents. (Cumming and Mayer, 1992). Free glochidia may attach to soft exterior parts of freshwater fish resting on the bottom (Fisher and Dimock, 2000). After a specific time, encysted larvae metamorphose into juvenile form. After this transformation, the juvenile become free and drop to the stream or lake bottom to begin an independent life resembling adult. The period of attachment varies from about 1 to 25 weeks depending on the host, location of attachment and water temperature (Barrington, 1967; Cumming and Mayer, 1992).

Compared to *Anodonta*, Pearl Mussel *Margaritifera margaritifera* is a short–term breeder, retaining its offspring only for 4 to 6 weeks during late summer in the marsupial. *U. crassus* also is a short–term breeder, but it produces eggs several times (up to five times) during its spawning season in spring.

Anodonta is a long-term breeder and the marsupia contain ripe glochidia from October until the end of the next May (Bauer, 2001). Since there is no information on reproduction time and larval development of *Anodonta cygnea* in Lake Cıldır and even from the other lakes in Turkey, this research seems useful and complementary. In this study, it was aimed to investigate the spawning time and larval development.

Material and Methods

In order to determine the reproduction time, *A. cgynea* samples were collected monthly by scuba divers in summer and both mussels and fish caught by gill nets under the ice layer in winter from May 2001 to July 2002 in Lake Çıldır were included into the study (Figure 1). It has long freezing period starting from December until the end of April. Ice layers may reach up to 110 cm depending on the duration of the cold season. All winter samples were carried to the laboratory in bottles and preserved in formaldehyde.

Length, height and convexity were measured with a caliper to the nearest 0.05 mm. Length was the maximum distance between the posterior edge and anterior edge, height was between dorsal edge and ventral edge, convexity was lateral form of shell (Zhadin, 1952; King, 1995). After the body measurements, samples were separated into 10 mm length groups containing 10 individuals in each. Specimens in the 10 mm length groups were observed monthly in order to determine the development of larvae. The length of larvae was measured under the stereomicroscope and photographed. Immersion oil and ammoniac were used in order to separate larvae and mucus. Moreover, the weight of empty and full gills, the length of larvae (glochidium), the number of larvae, and periods of gravidity were determined.

Marsupia were monitored and checked monthly for larval existence and for transition to parasitic stage. The total larvae content in a full marsupial gill was measured based on the weight of small marsupial piece (approximately 0.5 g) with larval mass and on the respective counting of partial amount of larvae. The larvae were counted by counting chamber under the stereomicroscope (Olympus Ch-2). The total number of larvae was estimated by the gravimetric method;

$$N = \frac{n.W}{g}$$

where the breakdown is as following; N: total number of larvae in the marsupia, n: number of larvae in the sample, W: total weight of larvae in the marsupia, g: weight of larvae sample,

All statistical analysis and graphs were done by using the software Microsoft Excel 7.0, Stats of Statistica 5.0. The mean and standard error (±SE) were calculated for all parameters in each group, and where necessary one-way analysis of variance (ANOVA) was used.

Results

The first larvae were observed in the gills at the end of July. Glochidia were expelled into the water next June. Larvae (glochidium) of *A. cygnea* were hosted by two species; *Cyprinus carpio* and *Barbus plebejus lacerta*.

Majority of the gravid mussels (with glochidium) were found in 75-125 mm length range n=733 (88%). The smallest gravid mussels were observed in the 55-65 mm length group and the maximum rate was reached (30%) in the 95-105 mm length group. Gravidity decreased as the length of mussel increased.

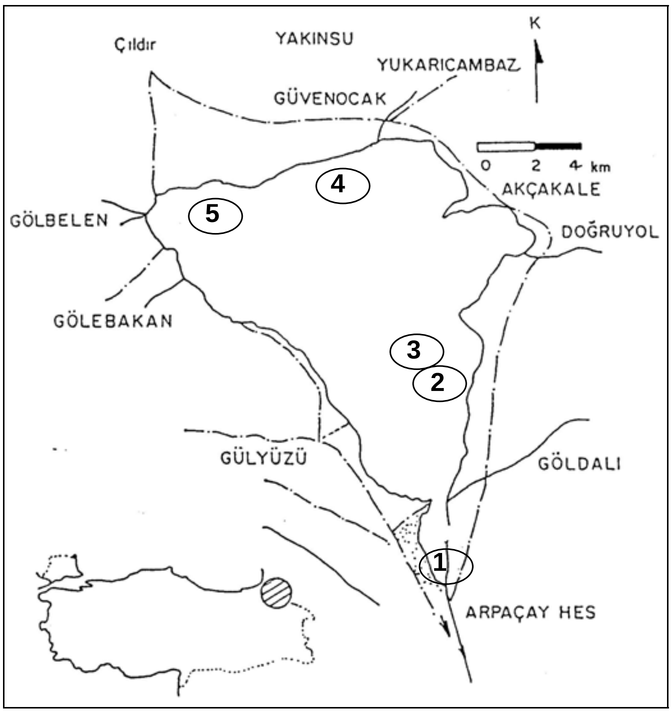

Figure 1. Sampling locations in Lake Çıldır.

The relationship derived between the number of larvae and mussel length is $N = 43.696L^{1.6764}$ and dry flesh weight is $N = 27916W + 36792$ (Figure 2A, B). The correlation coefficients are $r = 0.42$ and $r = 0.51$, respectively.

Samples greater than 55 mm all had glochidium and the following data was found as follows: the mean number of larvae was 114999±955, the mean shell length was 99.3±0.234 mm and the mean weight of larvae mass was 4.79±0.029 g (Table 1).

The relationship between length of *A. cygnea* and mass weight of larvae, length of mussel and the full gill were derived [($W = 0.0014\ L^{1.7476}$, r=0.58, P<0.05), ($W=0.0007\ L^{1.958}$, r=0.68, P<0.01)] (Figure 2C, D).

The relationship between the length and the height of larvae is HL = 0.2682 LL + 256.47, (r=0.23, P<0.05).

Larvae were yellow in colour; circle shaped, with 98.4±11.15 µm maximum diameter and motionless in june. In august, the colour of the larvae became darker; their shell was opaque, and their mean length was 271±8.36 µm. In september, larvae shape is under triangle form, and had also a hook. Larvae could open and close their shells, and the mean length

Table 1. Body measurements, weight, number of larvae, weight of full marsupia, weight of larvae mass by length groups for gravid mussel, (mean ± S.E)

Length groups	N	Length (mm)	Height (mm)	Convexity (mm)	Weight (g)	Number of Larvae	Full Marsupia (g)	Larvae mass (g)
55-64.9	1	59.2±0.1	34.5	17.8	15.3	13,378	0.72	0.66
65-74.9	1	74.3±0.1	38.3	25.1	35.5	78,600	3.81	3.24
75-84.9	4	81.98±0.2	44.6±0.13	27.63±0.201	48.91±0.52	103,654±5,342	5.28±0.179	4.45±0.185
85-95.9	10	89.6±0.2	45.1±0.07	30.02±0.111	62.8±0.27	96,776±2,041	5.44±0.068	4.16±0.05
95-105.9	4	102.8±0.3	51.1±0.15	33.63±0.369	89.2±1.45	171,012±7,990	7.37±0.232	6.01±0.23
105-115.9	8	109.1±0.2	55.4±0.10	34.6±0.13	97.39±0.75	113,672±4,444	5.85±0.116	4.46±0.127
115-125.9	4	119.9±0.4	60.9±0.22	39.05±0.294	125.6±0.57	92,748±3,118	7.44±0.109	5.61±0.138
125-135.9	2	126.9±0.4	63.1±0.38	42.05±0.038	157.02±1.69	235,592±5,853	10.80±0.069	8.65±0.031
Range		59.2-128.3	34.5-64.6	17.8-42.2	15.3-163.8	13,378-263,057	0.72-11.07	0.66-8.77
Mean±SE	34	99.3±0.23	50.83±0.11	32.5±0.079	83.14±0.49	114,999±955	6.11±0.032	4.79±0.029

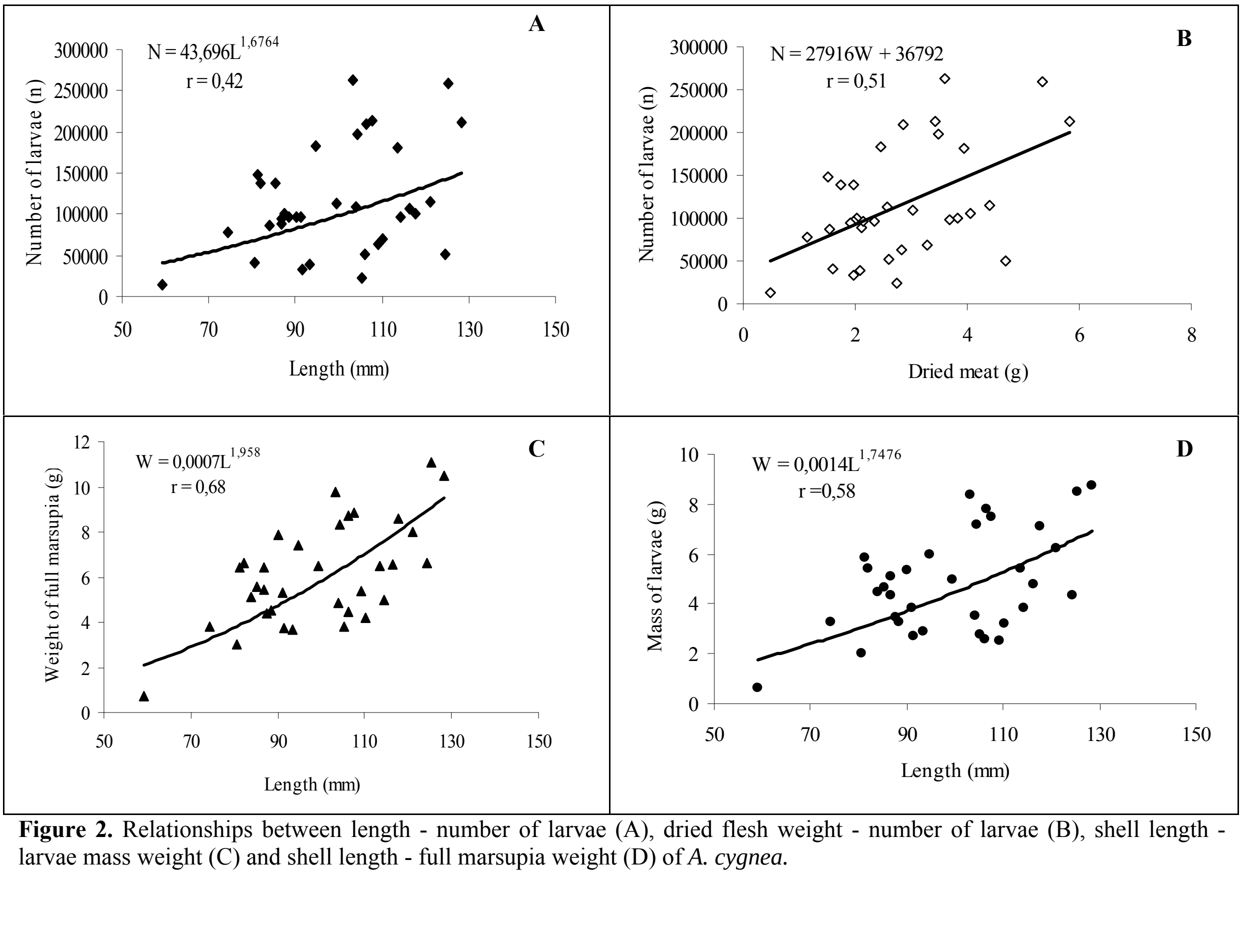

Figure 2. Relationships between length - number of larvae (A), dried flesh weight - number of larvae (B), shell length - larvae mass weight (C) and shell length - full marsupia weight (D) of *A. cygnea.*

reached to 359±4.115 µm, their height was 349±4.598 µm. In October, larvae had hooks and their mean length and height were 354±2.228 µm 353±2.869 µm, respectively, (Table 2).

Discussion

The time of release for Glochidia was found as the month of June. During the research period, the glochidia in marsupial had first been seen at the end of july. Larvae were carried in the marsupia until next June. It was concluded that all larvae had been released fully by the month of june because observations on marsupia showed that they were empty by this month. As a long-term breeder, marsupia of *Anodonta cygnea* contained ripe glochidia from october until the end of next may (Bauer, 2001). *Anodonta cygnea* have along gravidity period, lasting from august through the winter to march-april (David, 1999). In this research gravidity period has longer than the other study. The reason of longer gravidity may be due to hard climate conditions in the region, freezing of the Lake Çıldır surface from october to april, causing reduction of the metabolic rates.

The number of glochidia produced depends on both the size of glochidia and the size of the female mussel. They range from a few thousand to several millions. It shows differences depending on species. The number of larvae released by *A. cygnea* has a range from 3.1–3.7 x10^5 (Wächtler *et al*., 2001). In this research the mean number of larvae was found as 115,000. It is lower than as mentioned in the study done by Wächtler *et al*. (2001). The reason of this variation was attributed to differences in geographical site, ecology and mussel size.

The number of glochidia was in direct correlation with the shell length and the size of the marsupium, as is the case with the eutrophic lake (Bauer, 2001). Patzner and Müller (2001) studied the fertility of *A. cygnea* in lakes with different degrees of eutrophication. They studied the number of glochidia larvae of *Anodonta cygnea* in Lake Grabensee ($r=0.38$, $P=0.9$) and lake Mattsee ($r=0$, $P=0.9$).

In this research, we found significant differences about the relationship between the shell length and number of larvae ($r=0.42$, $P<0.05$), dry flesh and number of larvae ($r=0.51$, $P<0.05$)

Glochidium in the Unionidae, is in a more or less triangular, spherical or hat like shape and it is between 80-350 µ in diameter. Glochidium sizes can vary depending on the different species of unionides. (Wächtler *et al*., 2001). In their study, Wächtler *et al*. (2001) found *A. cygnea* which had a glochidium size of 310x310 µm. In another study, *Anodonta* sp. glochidium size was found as 0.34 µm (Bauer, 2001). Glochidium length sizes were found as 0.35-0.36 µm in the same study. Glochidium sizes showed differences according to different months and ranged between 50-425 µm (Table 2). Glochidium mean length size was found as 271±8.359 µm in August, 359±4.115 µm in September and 354±2.228 µm in October.

The relationship between the larvae length and larvae height was found as $r=0.23$, and it can be concluded that there were significant differences ($P<0.05$).

There was also a significant difference between the shell length and larvae mass ($r=0.58$, $P<0.05$); and the shell length and weight of full gill ($r=0.68$, $P<0.001$). The relationship between the shell length and larvae length was found as $r=0.15$.

The majority of known hosts of freshwater mussels are fish. Considering the long history of scientific interest in Unionidae, including their reproductive biology and ecology and both historic and renewed commercial importance of many species, relatively little is known about their hosts (Jansen *et al*., 2001). It was shown that there was a positive correlation between freshwater mussels and the abundance of fish species. Relationship between mussels and abundant fish species distributions has triangle (Vaughn and Taylor, 2000). In this study, glochidium were found on fish gills (*Cyprinus capio*, *Barbus plebejus lacerta)* on June 2002.

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Table 2. Larva sizes (µm) in months

	July (n=10)	August (n=10)	September (n=62)		October (n=97)	
	Diameter	Length	Length	Height	Length	Heigth
Range	50-150	210-310	312-380	250-425	325-370	275-425
Mean ±SE	98.4±11.15	271±8.36	359±4.12	349±4.60	354±2.23	353±2.87

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