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RESEARCH ARTICLE

Preliminary results on the growth of larval European lobster (*Homarus gammarus* (Linneaus, 1758)) in Turkey

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ABSTRACT

Sea lobsters are among the most valuable seafood traded commodities. Since its production with fishing has decreased every year in the world, many types of cultural studies have been carried out and success has been achieved. The aim of this research is to investigate the survival of European lobster (*Homarus gammarus*) larvae in Turkey and to determine the nature of the direction of growth opportunities. This research is the first study in Turkey investigating the nature of the direction of growth opportunities and the survival of European lobster larvae. Larval release, larvae feeding and survival possibilities were investigated on two egg-bearing lobsters obtained from Çanakkale coasts. Both broodstock larvae were able to survive until the post larval stage. The larvae of the first mature lobster reached 10.857 mm total length and 0.025 g live weight after approximately 30 days. The larvae of the second mature lobster reached 26.9 mm total length and 0.502 g live weight after 33 days. A significant difference was found in the larvae of two mature lobsters at the end of the experiment according to their initial dimensions in both length and weight ($p < 0.05$). In addition, it was determined that the growth was higher due to the higher temperature in the larvae of the second mature lobster.

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Introduction

FAO (2020) reported that world human population will have increased by about 34% until 2050, reaching to some 9.1 billion people. To be able to meet this increase with in nutrition, the current food output will have to augment by almost 70% of it. To equilibrate failure of the resultant supply to meet potential demand, protein sources from sea and freshwaters will be of vital importance in terms of feeding humans. Considering the present water sources under pressure of overfishing, likelihood to increase the concerned output is rather poor. Therefore, the most efficient supply for sources is aquaculture production of sea food which has been used through history (Jardas and Pallaoro, 1992). The species European lobster, *Homarus gammarus* has a region of distribution confined to the continent of Europe. The species has a wide geographic region over Atlantic Ocean in which it inhabits. Moreover, it spreads along the east coast of Europe from Sweden, Norway, Denmark, Lofoten Islands to UK, Ireland and Southern Morocco. Although it spreads less extensively, its presence has been determined along the Mediterranean and the Black Sea as well (Cobb and Castro, 2006: Prodöhl et al., 2007).

Considering global production of *H. gammarus* species, it was 3000 tons in 1950 increasing to 4800 tons in 1964 and in later years decreasing to 1739 tons in 1979 as the lowest value. However, it ranged from 4000 to 5600 tons following 2006 and reached to 4688 tons in 2018. Of European nations, Britain is the first by 3019 tons in the production from catching processes (FAO, 2020). The early records on trading lobster in Turkey date back to 1925 (21,888 kg). It was reported from Istanbul Fish Market that lobster was sold of 19,431 kg, 23,569 kg and 17,975 kg in 1921, 1922 and 1923, respectively (Deveciyan, 2011). Although production of lobster varied over the years, it increased to 60 tons in 1998 and in later years gradually decreased to 5 tons in 2018. Much of the catch has been obtained from the Aegean Sea and the rest from the Marmara Sea. It can be concluded that in recent years lobster production from catching processes has significantly decreased and import has been gradually increasing to meet the current demand for the product. In 2017, 38 tons of lobster in live and frozen forms (3,610,000 USD) were imported whereas Turkey's lobster export was only 1 ton (260,000 USD) in the same year (FAO, 2020).

It is clear that lobster populations in Turkey has been exposed to pressures caused by over fishing and other processes such as illegal fishing, pollution, degradation of habitat and predator pressure, etc. Therefore, studies and researches have to be conducted aquaculture processes for this species in Turkey. However, investigations are mainly focused on freshwater crayfish species in Turkey (Berber, 2005; Balık et al., 2006; Berber and Balık, 2009; Berber and Mazlum, 2009; Berber et al., 2010, 2011, 2012, 2019; Akhan et al., 2014; Türel et al., 2015; Türel and Berber, 2016; Berber and Kale, 2018). On the other hand, the studies on lobsters conducted are mostly related to those of species-specific artificial reefs (Acarli et al., 2018; Acarlı and Kale, 2020a, 2020b), taxonomy and reproduction biology concerning localities where the species is distributed in Turkey (Balkıs et al., 2002; Kocataş and Katağan, 2003; Bakır et al., 2014; Gönülal and Güreşen, 2014; Erkan and Ayun, 2014).

As for feeding difficulties in larval stages, cannibalism, and effects of environmental factors, rates of survival and growth for the species *H. gammarus* are observed to be low. Therefore, the present study aimed at determining and improving the growth stages of European lobster larvae in eggs and just after hatching processes and studying possibilities of their growth under controlled conditions.

Materials and Methods

The present study, which is the first performed study on the determination of larval stages of European lobster in Turkey, was conducted at Marine Life Research and Application Center at Dardanos, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University in Çanakkale from January 15 to May 11, 2015.

Two individuals of *H. gammarus* with eggs in their gonads were used, which are captured by fishermen off Karabiga, Çanakkale, in this study. They were transferred at the optimum conditions to the research center and separately placed into the two tanks of 500 L. The adults were fed with fresh fish and mussels, and leftovers siphoned from the feeding site. After eggs hatching out, free larvae were picked up using sieves and taken back into the tanks. Measurement of length and weight were made on lobster larvae on a daily with an electronic caliper. Larvae fed with enriched 0.5 L *Artemia* per day one. YSI Pro 2030 and WTW 3110 multimeters were used for temperature, dissolved oxygen, pH, and salinity measurements in the tanks.

Eggs of European lobster were taken to the laboratory in saline water without adding any fixative substance to avoid potential variation in diameters to measure and photograph them with no delay. Every ovum and its ovular diameter were measured and recorded. External capsules of some eggs were opened (exposed) using devices called pin wises to photograph embryo and organs, which was all performed by Olympus SZX7 stereo microscopy attached with v Q-Image Micro Publisher 3.3 RTV imaging program in the laboratory.

After adult lobsters with eggs in their gonads were placed on the study field, larval stages were determined from egg samples every three days based on development of water temperature. A

total of 20 eggs were taken from the different points of gonads attached to the abdomen every three days. Water in 75 cm diameter cylindrical conic tanks was arranged to be changed by 100% every three hours in the first 2 days then by 100% once a day. Tanks were cleaned every 24 hours. Larval density in the tanks were arranged to be 25-30 individuals per liter. For larvae feeding, green water technique of *Nannochloropsis sp.* in 400-800×10^{-1} cell/mL was provided and *Artemia salina* given to meet demand for nutrition as well. When phytoplankton was being entered, *A. salina* started to be given to the medium (5mL twice a day).

Statistical Analysis

Data were obtained for regression analysis and analyzed using one-way analysis of variance (ANOVA) to examine the effects of each passing day on the growth. Differences were considered significant at 0.05 significance level. All statistics analyses were evaluated using SPSS 19.0 statistical package.

Results

One of the physical properties of sea water used during the trial, temperature in particular was found to increase in larval hatching of the second adult with a significant impact on the larval development. Other properties were seen to be stable in the experiment during the study period (Table 1).

Developmental Stages of Embryos and Larvae

The egg size (width and length) and the embryo's eye size (length and width) are shown in Table 2. Table 2 also presents the measurements of length, width and values of eye size and width of the embryo.

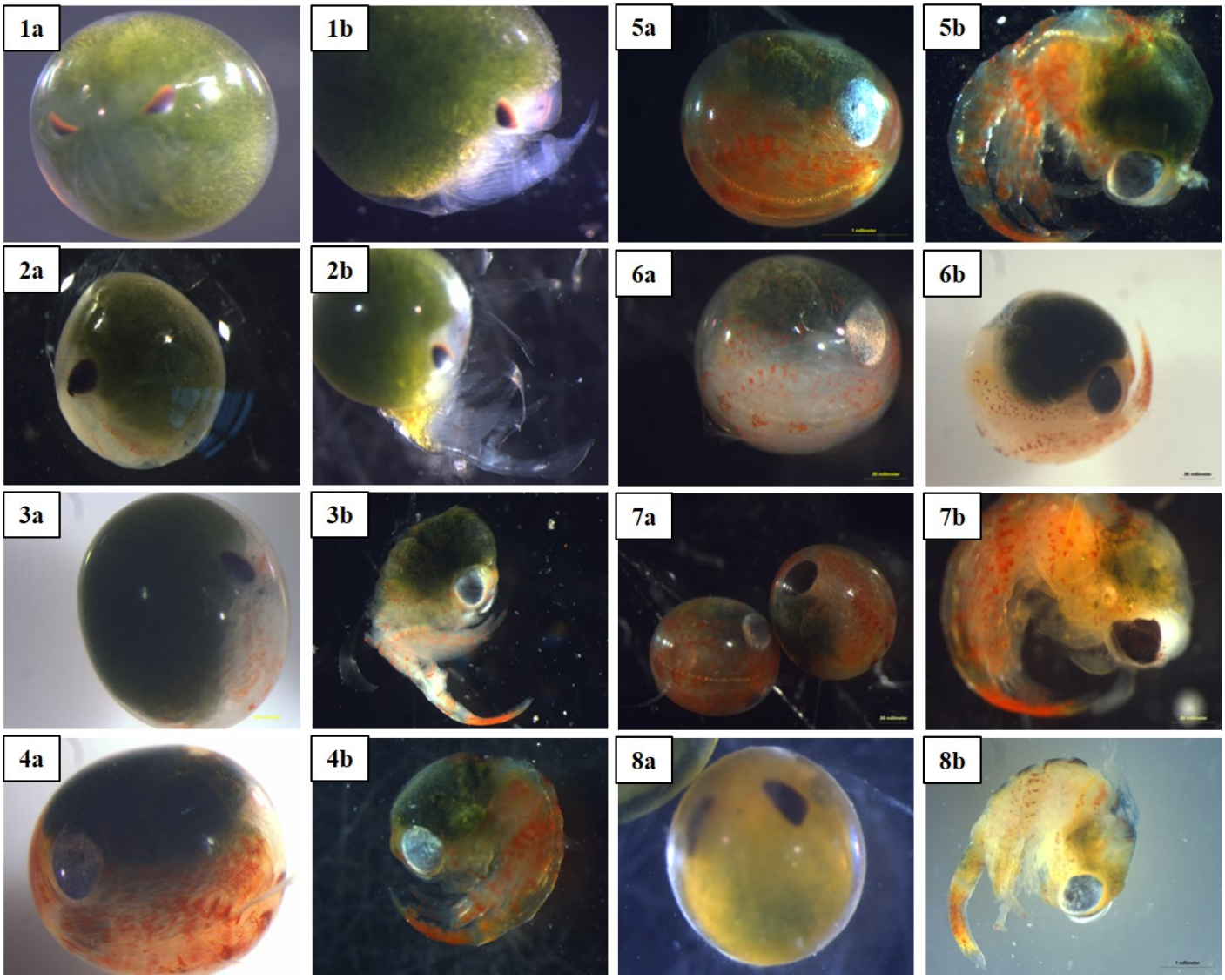

Figure 1. Embryonic development stages of *Homarus gammarus* green yolk 100% (1a-1b), consuming 20% of green yolk (2a-2b), consuming 30% (3a-3b), consuming 50% (4a-4b), consuming 60% (5a -5b), consuming 70% (6a-6b), consuming 80% (7a-7b), and the appearance of the larva that is about to hatch (8a-8b)

Figure 2. Larval development stages of *Homarus gammarus*; stage 1 (a), stage 2 (b), stage 3 (c), stage 4 (d) and juvenile (e)

Table 1. Measurements of temperature (°C), pH, dissolved oxygen (O_2) (mg/L) and salinity (S) (‰) in the experiments (SE: standard error)

Experiments (Duration)	Values	Temperature (°C)	Dissolved oxygen (O_2 mg/L)	pH	Salinity (‰)
Experiment 1	$\bar{x}$±SE	11.26±0.243	6.73±0.144	8.19±0.032	28.48±0.309
(25 days)	min-max	8.9-12.6	5.46-7.73	8.01-8.44	25.3-31.1
Experiment 2	$\bar{x}$±SE	12.96±0.131	7.24±0.145	8.55±0.056	28.27±0.223
(33 days)	min-max	12.4-13.4	6.79-7.83	8.37-8.72	27.3-29.1

Table 2. Measurements of length, width and values of eye size and width of the embryo (EW: egg width, EL: egg length, EEL: eye length, EEW: eye width; SE: standard error)

Experiments	EW±SE (µm)	EL±SE (µm)	EEL±SE(µm)	EEW±SE (µm)
Experiment 1	2.144±0.0056	1.974±0.0059	0.683±0.042	0.509±0.042
min-max	1.9-2.48	1.63-2.3	0.44-0.76	0.21-0.71
Experiment 2	2.165±0.033	2.452±0.035	0.709±0.028	0.497±0.017
min-max	1.99-2.31	2.3-2.648	0.609-0.838	0.408-0.548

Considering egg development stages, especially consumption of nutrition sac and its related color, a 30% consumption showed the sac with dark green color on it and its gradual consumption indicated a more visible body form with the eye turning from bright and light color to darker in tone (Figure 1, illustrations 3a and 3b). The egg membrane was torn apart to take the embryo out and to study its organelles. The process when hatching was about due showed the nutrition sac above the eye and preopod development was apparent. The abdomen was found to be in a visible extensional form and change to stage 1 in character when the hatching was due (Figure 1, illustrations 8a and 8b). The stages were examined by observing larval activities in the tank as well as microscopic examinations to establish development of larvae during the study. Accordingly, 4 larval and 1 juvenile stages were determined (Figure 2). For stage 1, pigmentation was the first characteristic in larval development in newly hatched individuals. Although the eye aperture did not grow in volume, variation was hardly observed to emerge in body length index until the first molting. Even if rostrum pointedness was not much, it was visible. Development of clamp was not strengthened yet (Figure 2a). In stage 2, coloration was seen to increase. Size of the eye was more obvious than in stage 1 and rostrum pointedness became clearer (Figure 2b). Development of clamps and preopods was found to be satisfactory enough to catch foods in suspension. Because pleopods and telsons did not sufficiently develop, larvae could not swim freely and suspended on water. Moreover, another significant characteristic at this stage is that development of clamp, preopod and telson enabled them to begin to swim on water and strengthening and deepening of clamp scissors emerged. Juvenile stage emerges until the period of time when growth, mating, spawning and incubation each has become part of annual cycle and those which has reached to this stage molt less frequently than previous stages. Individuals at juvenile stage hardly differ than adults. The front body was found to strengthen with visible hairs. Due to pointedness of rostrum, it was observed to elongate towards frontally in a way to effect body length (Figure 2c). At stage 4, mean carapace length, total length and weight were 3.75 mm, 12.6 mm and 0.0245 g, respectively. Individuals at post larval stage resembled adults but variously represented a stage of transition (Figure 2d).

Larval Growth

A significant increase was not found ($p>0.05$) when growth characteristics of larvae hatched on February 13, 2015 from the first adult until April 10, 2015 were examined (Figure 3) whereas those hatched from the second adult on April 15, 2015 showed significant differences in growth until May 18, 2015 ($p<0.05$; Figure 4).

Discussion

Temperature is widely known to have an impact on gonad and embryonic development of Crustacea species in the way it has on other living organisms (Acarli and Lök, 2009; Yildiz et al., 2011; Küçükdermenci and Lök, 2012; Acarli et al., 2015, 2018). Agnalt et al. (2013) reported that lobster development exhibits a positive relationship with temperature. Optimal water temperature is generally 20-22°C for *H. gammarus* species (Prodöhl et al., 2007). Moreover, lobster larvae are more tolerant to low temperatures than young or adult individuals. At 20°C larval period ends for about 20 days while it extents to 35 days at 15°C (van Olst et al., 1980). It was found that healthy larval development did not occur below 14°C. Schmalenbach and Franke (2010) reported that survival rate of *H. gammarus* larvae increased from 9% at 14°C to 80% at 22°C and its larval development decreased from 26 to 13 days.

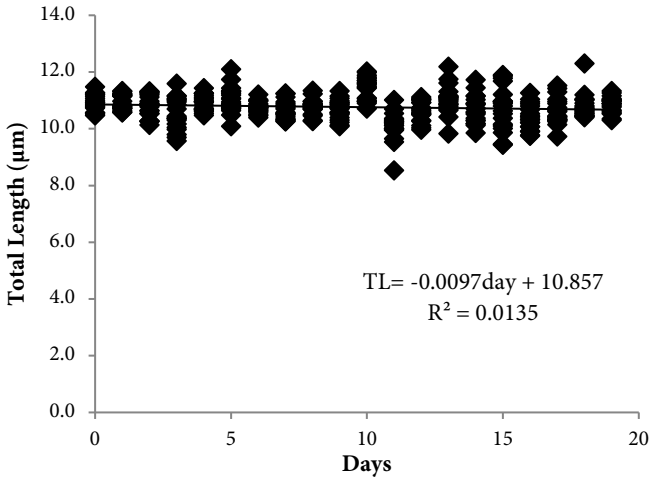

Figure 3. Larval growth of *Homarus gammarus* in the experiment 1

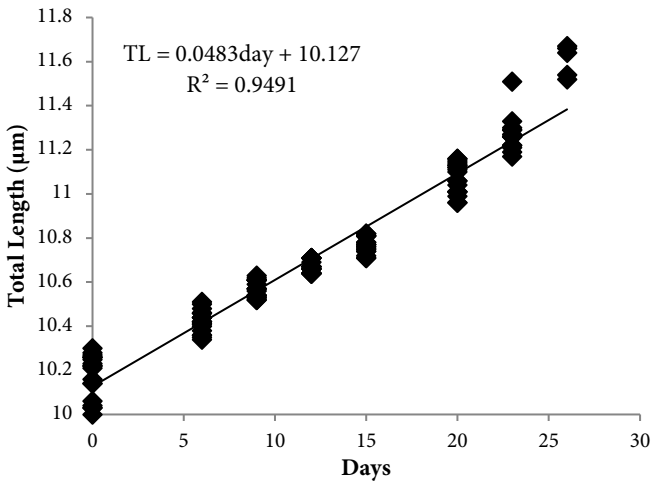

Figure 4. Larval growth of *Homarus gammarus* in the experiment 2

During the study, sea water filtered and fed to the system was used and no interference was made to increase temperature of water. Temperature of water was measured by 11.26°C and 12.60°C at the first and the second trials, respectively. The study showed that individuals could reach to juvenile stage for 33 days, expansion of which is believed to be temperature as the most effective factor. Although temperature below 14°C retarded larval development significantly, it still continued to develop. Schmalenbach and Franke (2010) reported that the molting did not occur under 10°C.

Molting in Decapods are affected by salinity, light density, social interaction, volume of habitat and water quality (Mikami and Kuballa, 2007). Considering the parameters below in terms of ideal water properties in studies on growing larvae, changes of salinity, pH and dissolved oxygen have been reported to have to be above 29-35‰, 7.8-8.2 and 8 mg/L, respectively (Burton, 2003). The lowest salinity tolerance in *H. americanus* was found to be 13.8‰ and 8‰ for larvae and young adults, respectively (Cobb, 1976). Under natural conditions, lobsters especially at larval stages do not prefer areas with salinity rate below about 20‰ (Fefer and Schetting, 1980). Low pH increases physiologic stress and affect individuals already under metabolic stress negatively (Agnalt et al., 2013). Salinity and pH of sea water used for larvae in the study are assumed to be at appropriate values for the organisms to growth.

Though food quality is considered an important factor which increases and controls productivity in decapod larvae, what is known is relatively little about food requirement and zooplankton for larval growth. One of the reasons for this is absence of an efficient nutrition which is acceptably digestible (Meyers, 1973, 1979; Eagles et al., 1986). For feeding larvae, European hatcheries uses minced fish, bivalves (Wickins and Beard 1991; Nicosia and Lavalli, 1999; Burton, 2003) and live baits such as *Artemia* spp. and *Acartia tonsa* as well as and wet or damp plankton preparations until recently (Fiore and Tlusty, 2005; Scolding et al., 2012). However, larval survival rate and growth rate of *Homarus* spp. is negatively affected especially when amount of nutrition has been insufficient in high density culture studies. One of the ways to reduce cannibalism to a minimum is to increase food density and thus prevent larvae starving much. In recent years, trials have been conducted to use ready-made feeds and rations prepared to meet content needed by larvae, which could not change importance of *Artemia* at all (Fiore and Tlusty, 2005; Powell et al., 2017). Their natural diets are composed of copepods and zooplankton as well as phytoplankton in less rate but feeds to be provided under culture conditions are supposed to have ability to produce high level of proteolytic enzyme. Since digestive enzymes of the carnivorous larvae are quite low, they have poor capacity to benefit from artificial feeds thus can feed on zooplankton such as copepods and *Artemia*. Recent developments in uses of micro capsules has enabled achievements to emerge in meeting nutritional requirements of penaeid shrimp larvae, which is promising in their uses for lobster larvae as well. The fact that recent developments in uses micro capsules have led to successful results for meeting nutritional requirements of penaeid shrimp larvae is promising in potential uses for lobster larvae as well (Meyers, 1973, 1979; Beal et al., 2002; Jørstad et al., 2005; Scolding et al., 2012; Drengstig and Bergheimb, 2013; Daniels et al., 2015). Evjemo et al. (2009) reported that larvae fed with formulated diets showed very poor development and were able to reach to stage 2 only after 20 days. The authors determined that *Artemia*-fed individuals entered stage 5 the same period with a survival rate of 91-94%. Lobster larva can ideally be fed with live *Artemia* but cannibalism occurs when given diets have been tasteless or insufficient (Wickins and Lee, 2002). It is known that *H. gammarus* generally have poor digestive enzyme activity. In other words, the species has very low stomach enzymes of trypsin and chymotrypsin though high activity of cathepsin L in their stomach fluid different from many other Decapod species, which has developed a strategy of keeping ingested foods long in the stomach to increase their digestion. High energy content and easily digestible food is needed to increase larval survival and growth rates in lobster aquaculture, in which context *A. salina* is also chosen as an important food (Kurmaly et al., 1990; Kumlu and Jones, 1997). At initial developmental stages of *Homarus sp*. larvae, *Artemia* nauplii is widely used. *A. salina* was employed as food in the present study. Individuals were grown until juvenile stage with length and weight from 10 mm to 25.60 mm and 0.023 g to 0.34 g, respectively.

In comparison with other lobster species, *Homarus* species including European lobster species are accepted as very resistant ones to thanks to their simple and short larval periods. However, production dynamics need to be comprehended well in order to be able to ideally manage present lobster stocks. Special feeding requirements are little understanding in larval survival and growth which are cited among the reasons for commercial inventorial fluctuations in the market.

Annually prepared and declared official statistics on sea foods indicate that they tend to decrease in parallel to current stocks due to output from catching processes. However, output amounts of species grown from aquaculture is observed to continuously increase. Similarly, production of marine lobster from fisheries is known to decrease every year. Decrease in natural stocks and necessity to protect natural sources, their high values of food and economics and employment potentialities if realized are among justifications for

aquaculture related to marine lobster. Although growth results from the present study are low as compared to those of other research, positive and promising signals exist under limited means. Environmental conditions such as temperature and abundance of nutrition tend to effect meroplanktonic larval development as well as distribution and quantity of populations (Kirby et al., 2007; Jackson et al., 2014). The conducted studies showed that regions where insufficient amount of food in the environment specifically have impact on survival and growth of Crustacean and fish larvae (Olson and Olson, 1989). On condition that Crustacean larvae have not sufficiently been fed, their hepatopancreatic cells would be irreversibly affected (Storch and Anger, 1983). Studies to be made further are supposed to focus on determining appropriate conditions for optimum output productivity and solutions to the problems of feeding at larval and juvenile stages.

Conclusion

In this study, the larval development stages of *H. gammarus* were investigated under two different temperature values. The results showed that high temperature has an increasing effect on larval development. The larvae of the first mature lobster reached 10.857 mm total length and 0.025 g live weight after approximately 30 days. The larvae of the second mature lobster reached 26.9 mm total length and 0.502 g live weight after 33 days. A significant difference was found in the larvae of two mature lobsters at the end of the experiment according to their initial dimensions in both length and weight ($p < 0.05$). All developmental stages of *H. gammarus* larvae were observed and they were able to survive until juvenile stage by the present study. This study is the first study in Turkey on the growth of *H. gammarus* larvae. The preliminary results of the present paper will encourage the further investigations on the subject.

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This paper is a part of MSc thesis of the first author.

Compliance with Ethical Standards

Authors' Contributions

Author SB designed the study, SA and AB wrote the first draft of the manuscript, performed and managed statistical analyses. EÖ worked in the experimental studies. All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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