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The Egg Production Rate of *Acartia* (*Acartiura*) *clausi* Giesbrecht, 1889 (Copepoda) in Sinop Peninsula (Southern Black Sea)

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Abstract

The egg production rate (EPR) of *Acartia* (*Acartiura*) *clausi* was measured between January and December 2008 in Sinop Inner Harbor (southern Black Sea). During the study period, EPR were found to be generally low varying between 2.04±0.43 (20 October) and 19.41±1.73 eggs/female.day (26 March). The mean EPR was recorded 8.62±1.02 eggs/female.day. The results have shown that water temperature and chlorophyll-*a* concentration are factors that affect the EPR of *A. clausi*; however, these factors are not always sufficient alone to explain the EPR in Sinop Inner Harbor.

Keywords: Fecundity, *Acartia* (*Acartiura*) *clause*, Turkey.

Güney Karadeniz'in Sinop Yarımadası'nda *Acartia* (*Acartiura*) *clausi* Giesbrecht, 1889 (Copepoda) Yumurta Üretim Oranı

Güney Karadeniz'in Sinop İç Liman bölgesinde *Acartia* (*Acartiura*) *clausi* türünün yumurta verimi oranları Ocak-Aralık 2008 tarihleri arasında ölçülmüştür. Araştırma süresince, günlük yumurta verimi oranı genellikle düşük olup; 2,04±0,43'dan (20 Ekim) ile 19,41±1,73 (26 Mart) yumurta/dişi.gün değer aralıklarında bulunmuştur. Ortalama yumurta verimi oranı 8,62±1,02 yumurta/dişi.gün olarak belirlenmiştir. Elde edilen sonuçlar, Sinop İç Liman bölgesinde su sıcaklığı ve klorofil-*a* konsantrasyonunun *A. clausi* türünün yumurta verimi oranını etkileyen faktörler olduğunu, fakat bunların her zaman kendi başına yumurta verimi oranını açıklamaya yeterli olmadığını göstermiştir.

Anahtar Kelimeler: Fekondite, *Acartia* (*Acartiura*) *clause*, Türkiye.

Introduction

Copepods play an important role in the vitality of fish populations (Runge, 1988). Discerning the various biological, physical and chemical factors that affect temporal and spatial variations in abundance and distribution, as well as the fecundity, growth, survival and mortality rates of copepods, is thus a basic objective of zooplankton ecology (Poulet *et al.*, 1995; Miralto *et al.*, 2002). Among these factors, determining the recruitment to copepod population (fecundity) is of particular importance (Ara, 2001).

Copepods play a key role in the marine ecosystem energy and nutrient cycle by forming a connection between primary (phytoplankton) and tertiary (planktivore fish) production (De-Young *et al.*, 2004). For this reason, the properties of the life cycle of copepod egg production and growth are vital information for any understanding of energy transfer in the marine pelagic food chain (Miralto *et al.*, 2002).

The Black Sea is enclosed, having only limited exchange with the Mediterranean. It is also a unique marine environment as the largest land-locked anoxic basin in the world. Until the mid-1970s, researchers considered the Black Sea to be a highly productive ecosystem at all trophic levels. Over the past 30 years, however, human manipulation of river outflows (Zaitsev, 1993), changes in nutrient loads (Bologa *et al.*, 1984), the introduction of exotic species (Mutlu *et al.*, 1994; Kideys and Romanova, 2001), excessive fishing (Gucu, 1997) and climatic variations (Oguz, 2005a) have meant drastic changes in the Black Sea ecosystem (Sorokin, 1983; Niermann *et al.*, 1994; Oguz, 2005b).Copepods, in particular, have been deeply affected by these changes. Through the early 1990s, *A. clausi* remained successful in both coastal and open habitats as the copepod least affected by these unfavorable conditions, and accordingly became the dominant copepod species, comprising up to 85%

of the total fodder zooplankton biomass in Black Sea coastal regions (Kovalev *et al.*, 1998a).

Copepod studies began 150 years ago with the identification of the species, but studies of Black Sea copepod reproduction have been limited. Most fecundity studies have been of *Calanus euxinus* Hulsemann, 1991 in the northern Black Sea (Greze and Baldina, 1967; Sazhina, 1996; Arashkevich *et al.*, 1998; Ostrovskaya *et al.*, 1998; Besiktepe and Telli, 2004). To fill this research gap, we seek to determine the egg production rate of *A. clausi* and found among the area's plankton population throughout the year (Ünal, 2002; Üstün, 2005; Bat *et al.*, 2007; Ustun *et al.*, 2007). No such study has heretofore been undertaken.

The Black Sea population of *A. clausi,* an epiplanktonic, neritic copepod, is found in coastal areas to as deep as 50–70 m below sea level (Kovalev *et al.*, 1998b; Hubareva *et al.*, 2008). The species shows limited migration activity in the Black Sea because of its daily feeding rhythm (Besiktepe, 2001). It is an important food source for anchovy, sprat and pelagic fish (Tkach *et al.*, 1998; Berdnikov *et al.*, 1999). The local population of this species is affected by temperature, salinity, and the highly variable availability of food (Stalder and Marcus, 1997; Gaudy *et al.*, 2000; Chinnery and Williams, 2004). This species no carrying eggs sacs, depositing eggs freely release into sea water (Sazhina, 2006). After mating, *A. clausi* lays eggs freely in the water, 15 times in one generation period (almost 16 eggs each time), and 7 times in the Black Sea (Greze and Baldina, 1967).

Therefore it is essential to calculate the egg production rate of *A. clausi* in laboratory conditions. Determining the relationship of these results with the temperature and chlorophyll-*a* concentration is beneficial for marine biology.

Materials and Methods

Sampling Area

Sinop is located in the Boztepe Peninsula, which stretches towards the northern coastal line of the southern Black Sea. In ancient times, this area earned the name *Pontus Euxeinos* (inhospitable sea) based on its reputation for fierce waves and storms (Işık, 2001). Sinop, with its 175 km coastal line, assume importance in the region thanks to its natural harbor, offering anchorage protected from northern and eastern winds (Anonymous, 2011).

The city is important geographically, sitting at an important crossroads connecting the peninsula to the mainland. The peninsula, which stretches east, makes the research area the only coastal area of the Black Sea Region that faces south. Furthermore, its many-caved coastis the most sinuous of the Black Sea, and it is shallow, having a maximum depth of 50–55 m (Anonymous, 2011). Its many Zostera grassbeds are protected from heavy wave action, and accommodate benthic, demersal, and pelagic organisms as well as otters (Aysel *et al.*, 2004; Dural *et al.*, 2006).

July through September, winds from the east that blow for more than two days occasion a phenomenon known as "upwelling" or "sea cold" which enriches the water's nutrient content, attracting certain organisms, such as *Pseudocalanus elongatus* and *Calanus euxinus*. Meanwhile, the sudden cold finds many demersal and semi pelagic fish species coming to the coast to faint. Sinop, at the meeting point of east- and west-flowing currents, thus sits near an unusually rich marine biological environment, enhanced by the city's low population and underdeveloped industry, which produces little pollution.

Sinop and its surroundings are an important gateway and waiting area for anchovy, horse mackerel, bluefish and similar migrant fish, giving the area rich and important fish stocks. This productive fishing center and the coastal area are also increasingly used for recreation.

The zooplankton sampling to determine the EPR of *A. clausi* was performed in the station in Sinop Inner Harbor (42°00'21" N - 35°09'32" E; depth of 50 m) between January and December 2008 with an interval of 2 weeks periods (Figure 1). The samples were collected with Standard plankton net (mesh size= 112 µm, mouth diameter =50 cm) from the bottom to the surface (50-0 m) by vertical tows. The sampling was carried out during cruises of R/V "Arastırma I" of Sinop University Fisheries Faculty.

Measurement of Environmental Parameters

The temperature of the sea water was measured with U-10 Horiba and an YSI 6600 brand Water Quality Measurement. In order to determine the chlorophyll-*a* concentration, GF/C filters that have 0.45 mm mesh size have been used. Sea water was filtered in these filters and measured in 90% acetone extracts with HEYIOS Thermo-Spectronic brand spectrophotometer (APHA, 1992). Chlorophyll-*a* concentration was used as phytoplankton index in this study.

Determining the Egg Production Rate

Collected individuals were put into a container filled with diluted sea water. The surface water was collected at the time and place of the capture of organisms. After collection, these samples were transported to the laboratory as soon as possible.

The plankton material that was brought to the laboratory was filtered with plankton net of 300 µm mesh size. In this way small individuals were sent away and the adult *Acartia* individuals were collected easily. The healthy and adult females among the alive plankton samples were selected randomly under the microscope by using a wide-mouth pipette. The selected individuals were placed one by one in the culture containers filled with sea water (10 ml) (filtered in 20 µm mesh in order to keep the metazoan zooplankton and other copepod eggs away).

Incubation took place in 24 hours in sea water temperature and under natural daylight. Adult females were not fed during experiments. After 24 hours the eggs produced were counted with a stereo microscope.

The fecundity rate was calculated by taking the average of the eggs produced by the female in 24 hours observation period (egg/female.days). All females that laid eggs and did not lay eggs were included (Runge and Roff, 2000).

After the experiment, the contents of each container (adult female, egg, empty shell, naupli) were kept in bottles full of 4% buffered formaldehyde (Ara, 2001; Miralto *et al.,* 2002; Pagano *et al.,* 2004).

Statistics

In statistical analysis, SPSS 17.0 program was used. Egg production rate was taken as dependent variables. It matched with water temperature and chlorophyll-*a*. Linear regression analysis and curve fit cubic regression analysis were performed. The relationship between dependent and independent variables was assessed with Spearman Correlation Test (one-tailed). The results were evaluated between 95% confidence interval and between P<0.05 and P<0.01 significance level (Zar, 1984).

Results

Environmental Parameters

During the sampling period, monthly variations in water temperature and chlorophyll-*a* concentration at the surface are given in Figure 2. Water temperature varied from 5.7°C on 25 January to 27.2°C on 19 August. Chlorophyll-*a* concentration values ranged from 0.026 to 1.161 mg/m^3. Chlorophyll-*a* concentration was low from July to the beginning of September, and was high from January to April.

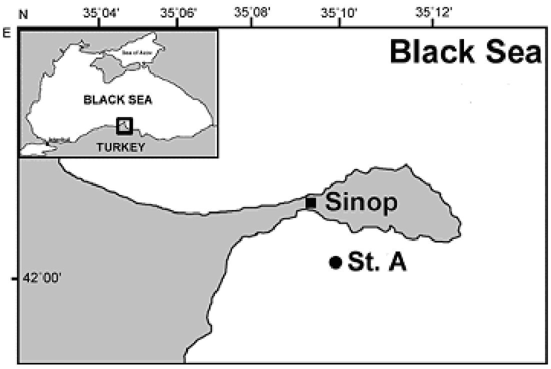

Figure 1. Location of sampling station (St A).

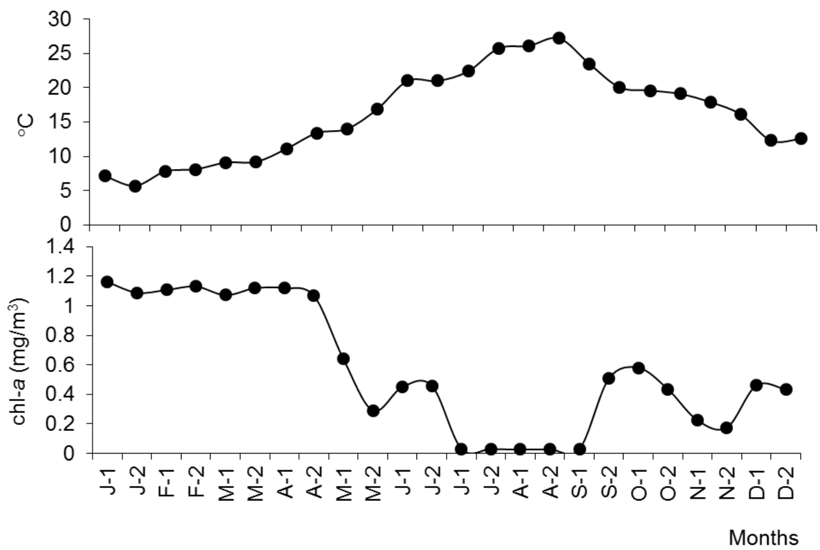

Figure 2. The monthly change of temperature (°C) and chlorophyll-*a* concentration (mg/m^3) at the surface water between January-December 2008 in Sinop coastal area.

Determining the EPR of *Acartia* (*Acartiura*) *clausi* (Copepoda)

The egg production rates showed important seasonal fluctuations and was characterized by three peaks (early and late spring and early winter) (Figure 3). The EPR of *A. clausi* estimated by the incubation experiment were low in October-December period, and were high from March to May. The EPR fluctuated from 2.04±0.43 eggs/female.day on 20 October to 19.41±1.73 eggs/female.day on 26 March (Figure 3 and Table 1). The mean EPR was measured 8.62±1.02 eggs/female.day.

Effects of the Environmental Factorson the EPR of *Acartia*(*Acartiura*) *clausi* (Copepoda)

In the study, the highest EPR was found in spring months in 8.6-17.5°C; and the lowest values were observed in autumn months in 16.94-23°C. A negative correlation was determined (r: -0.375, p:0.035, P<0.05) between the EPR and water temperature (a weak relationship in reverse way). The regression equation that shows the linear regression analysis of EPR and water temperature is: EPR= 14.206-0.355°C (r:0.427; p:0.037, P<0.05). However, in curve fit test, this distribution is observed in accordance with cubic regression curve. EPR= 7.614°C-0.529°C^2+0.011°C^3-21.501 r:0.593, p:0.031, P<0.05) (Figure 4).

The highest EPR was determined 0.64-1.12 mg/m^3, and the lowest one was estimated0.18-0.43 mg/m^{3}in chlorophyll-*a* concentrations .During the study, the chlorophyll-*a* value was calculated as mean 0.57±0.088 mg/m^3. A positive and statistically meaningful correlation was found between chlorophyll-*a* concentration and the EPR (r:0.498, p:0.007, P<0.01). The regression equation that shows

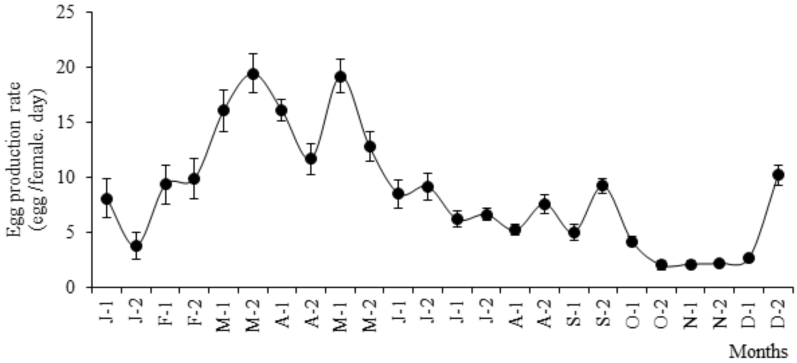

Figure 3. The monthly change of the EPR of *A. clausi* between January-December 2008 in Sinop coastal area.

Table 1. The summary of the experimental study of the EPR of *A. clausi*

Date	Temperature during experiment (°C)	Number of total females	Females that lay eggs	% Females that lay eggs	Mean egg/female/day±SD
22.01.08	7.6	28	15	53.57	8.07±1.76
25.01.08	5.6	25	9	36.00	3.76±1.24
26.02.08	9.6	40	18	45.00	9.33±1.79
29.02.08	8	39	19	48.72	9.85±1.82
11.03.08	10	34	27	79.41	16.03±1.86
26.03.08	8.6	49	42	85.71	19.41±1.73
16.04.08	10.6	48	44	91.67	16.04±0.99
30.04.08	11	43	31	72.09	11.67±1.41
08.05.08	11.2	49	45	91.84	19.16±1.56
30.05.08	17.5	39	33	84.62	12.77±1.30
18.06.08	20.1	30	24	80.00	8.5±1.29
24.06.08	20.5	26	21	80.77	9.15±1.19
08.07.08	21	37	30	81.08	6.19±0.71
22.07.08	24.4	35	31	88.57	6.63±0.55
12.08.08	25.7	40	35	87.50	5.2±0.50
19.08.08	26.2	39	33	84.62	7.59±0.84
09.09.08	23	43	31	72.09	5±0.69
23.09.08	19.2	50	48	96.00	9.2±0.67
15.10.08	19.2	51	42	82.35	4.18±0.44
20.10.08	19	51	23	45.10	2.04±0.43
05.11.08	16.94	60	33	55.00	2.13±0.29
21.11.08	17.3	60	38	63.33	2.22±0.28
16.12.08	13.1	51	38	74.51	2.67±0.31
22.12.08	12.4	59	51	86.44	10.19±0.88

the linear regression analysis with chlorophyll-*a* and EPR is as follows: EPR= 5.154+6.095 chl-*a* (r:0.507; p:0.011, P<0.05) (Figure 5).

Discussion

Zooplankton growth and production rates fundamentally affect the population dynamics of marine organisms given zooplankton's important role in transferring energy produced on the primary level of the zooplankton food chain to creatures in the chain's upper level. We studied the relationship of temperature and chlorophyll-*a* concentration to the egg production rate (EPR) of *A. clausi* from January to December 2008 in the Sinop Region.

It has been determined that sea temperatures and phytoplankton biomass undergo significant seasonal changes in the Sinop coastal area, with surface sea temperatures ranging from 7.1°C to 27.2°C in 2002–2003. In 2002, chlorophyll-*a* values ranged from 0.02 to 0.97 mg/m^3(mean: 0.37 mg/m^3), and from 0.2 to 2.2 mg/m^3(mean: 0.6 mg/m^3) in 2003. Phytoplankton biomass was found to shrink significantly in winter. Dinoflagellat species (especially *Protoperidinium* sp.) dominated in winter while diatom species (*Pseudonitzschia* sp.) were more pervasive in spring and summer during that same period (Bat *et al.*, 2005; Sahin, 2005). Our study found temperatures ranging from 5.7-27.2°C, while chlorophyll-*a* values varied from 0.026 to 1.161 mg/m^3 (mean: 0.58 mg/m^3).

A. clausi reproduce all year in the Black Sea, with maximum abundance of the early stages (egg and naupli) observed in late spring, early summer and autumn (Ünal, 2002; Üstün, 2005). Ünal (2002) found egg-laying in the Sinop coastal area to be at its peak in September 1999. We observed a high rate of *A.clausi* reproduction in the spring, but lower values in the autumn, except for September. In the northern Black Sea, Sazhina (1996) observed an *A. clausi* fecundity rate of 5–15 eggs/female.day at 20–22°C in September 1992, and 8-45 eggs/female.day at 14°C over a 24-hour period in May 1992. The mean EPR of *A. clausi* was determined to be 22.0±7.6 eggs/female.day in the northern Black Sea, with a recorded EPR of 1–22 eggs/female.day at ~21°C in September; and 2–42 eggs/female.day at ~14.41°C in May in the Sinop coast. The mean EPR of those two months was calculated at 11.5±1.05 eggs/female.day. Those EPR value ranges were similar for the north and south Black Sea. However, the average EPR value was higher than our finding. The fact that Sazhina (1996) studied more stations than we did may explain this difference. The maximum EPR obtained for *A. clausi* in the present study (42 eggs/female.day)

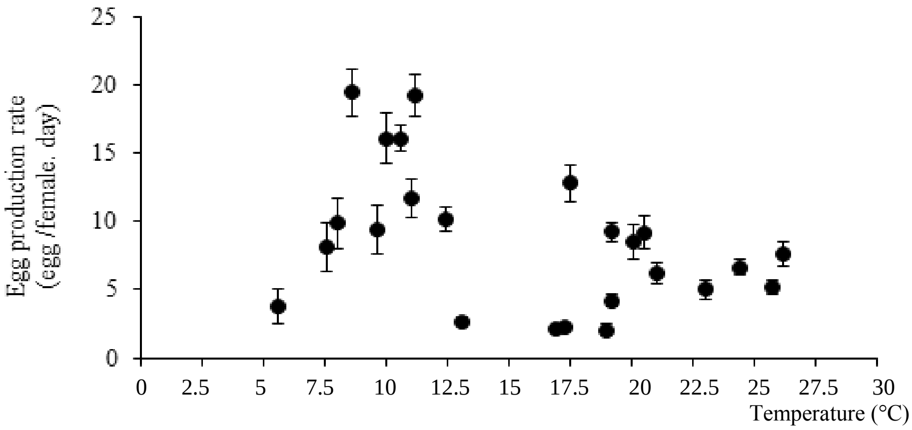

Figure 4. The relationship between the EPR of *A. clausi* and water temperature (°C).

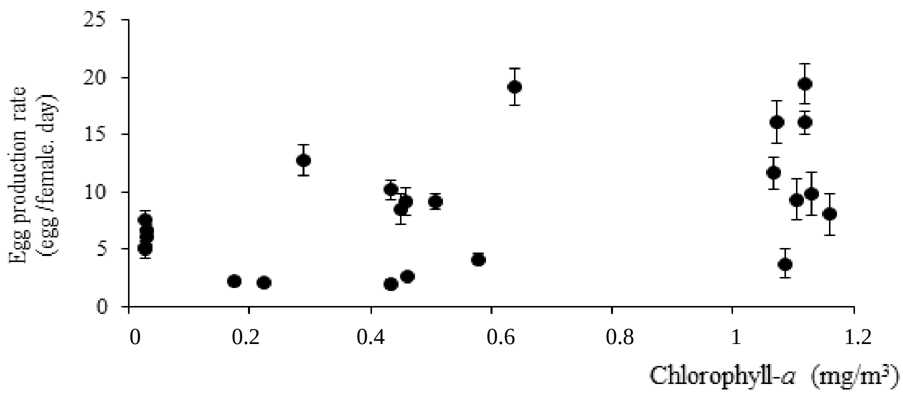

Figure 5. The relationship betwe en the EPR of *A. clausi* and chlorophyll-*a* concentration.

was similar to values recorded by Ianora and Scotto di Carlo (1988). The mean minimum and maximum EPRs in Ebrié Lagoon (Pagano *et al.*, 2004) were higher than those of other regions (Table 2).

Copepod EPR is influenced by such endogenous factors as gonad development stages (Eisfeld and Niehoff, 2007), and by such dominant environmental factors as salinity (Castro-Longoria, 2003; Chinnery and Williams, 2004; Uriarte and Villate, 2006), photoperiod (Uye, 1980), turbulence (Kiørbe and Saiz, 1995; Gutiérrez *et al.*, 1999), temperature, and the abundance and quality of food (Poulet *et al.*, 1995; Chaudron *et al.*, 1996).

Phytoplankton concentration (the amount of nutritious elements) is generally determined by chlorophyll-*a* concentrations in the area. Some studies have observed a strong relationship between copepod EPR and chlorophyll-*a* concentration (Landry, 1978; Uye, 1981; Kiørboe and Nielsen, 1994; Jung *et al.*, 2004; Pagano *et al.*, 2004). However, other studies have recorded no such relationship (Ianora and Scotto di Carlo, 1988; Ianora and Buttino, 1990; Gutiérrez and Peterson, 1999).

We found a weak relationship between *A. clausi* EPR and chlorophyll-*a* concentration (r:0.498, P<0.01). This finding may be explained by the very low chlorophyll-*a* concentration values during the sampling period (0.026-1.161 mg/m^3). The weak correlation observed on many occasions shows that copepod EPR may be highly related to food quality (Jónasdóttir and Kiørbe, 1996; Ianora *et al.*, 1996; Miralto *et al.*, 2002) and to the size, type, shape, and chemical content (protein, oil acids, etc.) of food (Jónasdóttir *et al.*, 1995) rather than to total chlorophyll. The food taken in must balance the great energy that egg production requires. When phytoplankton biomass is relatively low, however, *A. clausi* can feed on micro-zooplankton and detritus, thus masking this relationship. Such circumstances may also play an important role in cannibalism (Richardson and Verheye, 1998). Hence, one may expect a poor correlation between EPR and chlorophyll. In this study of the Black Sea, total chlorophyll-*a* concentration did not emerge as a good predictor of fecundity.

Another important environmental influence on EPR is temperature, and some studies have observed a relationship between temperature and *A. clausi* EPR and egg development (Landry, 1978; Sekiguchi *et al.*, 1980; Uye, 1981; Castro-Longoria, 2003). However, some studies have found that temperature in and of itself does not greatly affect EPR (Huntley and Lopez, 1992; Hay, 1995). Hay (1995) found that *A. clausi* EPR was not related to temperature and chlorophyll-*a* concentration in the North Sea, while Halsband-Lenk and Hirche (2001) showed that temperature influences egg production rate more than North Sea feeding conditions.

Our study found a negative correlation between EPR and water temperature (r: -0.375, p:0.035, P<0.05). The highest EPR was at 8.6–11.2°C (in spring months), and the lowest at 17–19.2°C (in late October, November and in early December). We observed that when temperatures were higher, *A. clausi* showed reduced EPR (Fig 2 and Fig 3). Ara (2001) made a similar observation for *A. lillijeborgi*, and argued that the finding may be explained by such physical and physiological activities as swimming, respiration and excretion.

Our results show that water temperature and chlorophyll-*a* concentration affect *A. clausi* EPR in the Sinop Inner Harbor; however, these factors are not always sufficient on their own to explain EPR. The weak correlation between *A. clausi* EPR and temperature and chlorophyll-*a* may be due to environmental and geographical conditions, changes in the community structure of phyto-zooplankton, laboratory conditions, or variations among individuals of the species (age, the size of female, fertility, transport stress).

Table 2. Daily egg production rates (EPR, eggs/female.day) of *A. clausi* in various regions

Region	Min-Max Mean EPR	Max ind. EPR (Temperature)	Temperature (°C)	Chlorophyll-*a* (mg/m^3)	References
Jakle's Lagoon, U.S.A.	7-25	70 (20)	ND	ND	Landry, 1978
Bedford Basin, Canada *	1.8-20.4	ND	2.4-19.9	ND	Sekiguchi *et al*., 1980
Gulf of Naples, Italy	ND	41 (15)	ND	0.39-2.99	Ianora and Scotto di Carlo, 1988
Bahía Magdalena, México	ND	11.5	19.8-21	0-5.8	Gutiérrez *et al*., 1999
Helgoland Roads, Southern North Sea	ND-33.5	57	0.5-20.2	ND	Halsband-Lenk and Hirche, 2001
Northern Adriatic Sea	2.7-13	ND	ND	ND	Miralto *et al*., 2002
Solent-Southampton Water system, south coast of England	1.8-14.3	ND	5-20	ND	Castro-Longoria 2003
Ebrié Lagoon, south of Côte d'lvoire	10-60.3	ND	25.8-31.5	6.7-30.5	Pagano *et al*., 2004
Helgoland Roads, North Sea	18-28	132	5-7	ND	Eisfeld and Niehoff 2007
Sinop coastal, Turkey	2.04-19.41	42 (11.2)	5.6-26.2	0.026-1.161	This study

* *Acartia clausi hudsonica*
(ND: No Data)

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