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EFFECTS OF ALKALINITY ON FECUNDITY OF *Caligus minimus* PARASITIZING ON SEABASS, *Lates calcarifer* IN LABORATORY CONDITIONS

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Abstract: Caligid copepod is one of the most common parasites in marine fish culture, causing negative effects on growth and survival of its host. This study aims to assess the fecundity of *Caligus minimus* parasitizing on seabass (*Lates calcarifer*) and evaluate the parasite's abundance dynamics under various alkalinity levels. Wild *C. minimus* were recorded with 60.9 ± 14.3 eggs in its egg string, which varies in length (3.38 ± 0.27 mm), and egg sizes ranging from 0.34 mm (width) to 0.08 mm (length). Wild *C. minimus* were observed to attach and survive on its host for 50 to 71 days, producing one to three egg strings in its life. Under 60 ppm and 70 ppm of alkalinity condition, the abundance and hatching index of *Caligus* reached a peak of 734 ± 14.5% and 919 ± 25.5% of the initial infection. There were no records of hatching at 10, 20, 110 and 120 ppm of alkalinity. Within 21 days, no significant difference in the number of egg and egg size under various alkalinity levels were observed. These results demonstrated that alkalinity strongly affects the population of caligid parasites.

KEYWORDS: Fecundity, *Caligus minimus*, *Lates calcarifer*, alkalinity

Introduction

Sea lice (copepods) have been reported as one of the most critical problems in marine fish culture, causing severe negative effects on stock survival, growth and susceptibility to disease (Johnson *et al.,* 2004; Skovgaard, 2005; Maran et al., 2009). *The ensuing losses from fish deaths as a direct or indirect consequence of parasitic copepod infestation may be huge* (Torrissen *et al.,* 2013; Lafferty *et al.,* 2015). Sea lice are also known to rapidly reproduce and survive in large populations (Tully *et al.,* 1993), which may affect wild fish populations (Krkošek *et al,* 2005; Costello, 2009).

Copepod infestation, especially by *Caligus minimus*, is common in southeast Asian seabass (*Lates calcarifer*) and has also been reported in its Mediterranean cousin (*Dicentrarchus labrax*), causing losses to fish farmers there (Khoa *et al.,* 2018; Noor El-Deen *et al.,* 2013). The parasites will damage the host's skin and epidermal layer, leading to stress, osmotic problems and secondary infections (Johnson *et al.,* 2004; Er & Kayiş, 2015; Saraiva *et al.,* 2015; Khoa *et al.,* 2018). Shinn *et al.* (2015) reported that 80% of seabass farms worldwide have been affected by this parasite, which resulted in a 30 to 50% loss of stock. However, the impact of parasitism was limited only to economic and environmental aspects (Heuch *et al.,* 2005).

In Malaysian fish farms, the infestation is also a prevalent problem. Among the reported species of parasites and their hosts are C. chiastos in Lutjanus johni, *C. longipedis* in *Gnathonodon speciosus; C. rotundigenitalis* in *L. erythropterus; C. epidemicus* in *L. calcarifer* and *Epinephelus coioides;* and, *C. punctatus* and *C. mimimus* in *L. calcarifer* (Maran *et al.,* 2009; Leaw *et al.,* 2012; Muhd-Faizul *et al.,* 2012; Khoa *et al.,* 2018).

The impact of environmental factors on parasite population dynamics are rarely documented. Previous studies had observed the reproduction and life cycle of copepods (Lin & Ho, 1993; Piasecki & MacKinnon, 1995; Heuch *et al.,* 2000; González & Carvajal, 2003; Khoa *et al.,* 2018) or created a model population based on fecundity (Tully & Whelan, 1993; Bravo *et* *al.,* 2009). A good understanding of how the environment affects parasite population may come in handy in predicting outbreaks and managing the disease they cause.

Tully & Whelan (1993) suggested that the fecundity of copepods was decisively associated with water temperature. The body size of female lice had been observed to increase at low temperatures, but egg development took longer and had low hatching index (Nordhagen & Schram, 2000; Heuch *et al.,* 2000). The parasites found on wild fish were larger and had longer egg strings compared with those on farm fish (Tully & Whelan, 1993; Nordhagen & Schram, 2000). Nevertheless, the direct effects of environmental factors were not clear.

Being Crustaceans, caligid copepods require significantly high levels of alkalinity in their environment to harden their new exoskeletons after moulting (Middlemiss *et al.,* 2016). The alkalinity level and Ca^{2+} in water had been shown to play an important role in post-moulting calcification of crustacean exoskeletons, which affects growth performance and survival rate of these organisms (Cameron, 1989; Truchot & Forgue, 1998; Middlemiss *et al.,* 2016). Interruptions to the post-moulting process significantly could significantly reduced the population and productivity of crustaceans (Cowthorn, 1997; Borisov *et al.,* 2007; Middlemiss *et al.,* 2016). However, most studies were conducted on large and economically-important crustaceans, such as lobsters (Middlemiss *et al.,* 2016), crabs (Cameron, 1989) and shrimps (Furtado *et al.,* 2014), and none was ever conducted on copepod parasites.

Therefore, this study aims to evaluate the effects of alkalinity on the fecundity and population dynamics of *C. minimus* parasites found in *L. calcarifer*.

Materials and Methods

Sample collection and fecundity study

Sampling was undertaken every four months throughout the year (June, October, February) with 150 infected seabass sampled. *C. minimus* infested *L. calcarifer* were collected from fish farms in Bukit Tambun, Penang, Malaysia (5°16'01.83N and 100°27'26.74E) and transferred to the Parasitology laboratory at University Malaysia Terengganu in Kuala Terengganu, Terengganu, Malaysia.

The parasites were removed from the host and observed under a microscope to identify them according to the methods by Boxshall & Defaye (1995). A total of 100 *C. minimus* ovigerous females were isolated and reared in separate beakers containing 50 ml of seawater to collect the copepodids they produced. The copepodids were then introduced to seabass fingerlings in small aquariums until the females matured and produced egg strings.

The new copepodids' lifespan, egg string length, egg size and number of eggs were measured and calculated according to Heuch *et al.* (2000) and Schram & Heuch (2001). The total length of egg strings was averaged from 100 randomly-selected egg strings. For the size of a single egg, the length of 10 egg sections in each egg string were measured under the microscope and averaged. The number of eggs was calculated by dividing the total length of egg strings with the length of the egg sections as shown in Equation 1 (Heuch *et al.* 2000).

$$\text{Number of eggs per female} = \frac{\textit{Total length of egg strings}}{\text{length of single egg}} \quad (1)$$

Hatching index at various alkalinity levels

The experimental laboratory set-up was described in Figure 1. Ovigerous C *minimus* females with mature eggs were placed in three 50 ml beakers. Each beaker was stocked with 10 parasites. Before stocking, the eggs were counted to determine the percentage of viable and non-viable eggs. The latter appeared dark when illuminated from below, were irregularly packed or merged into one long section in the egg string as described by Heuch *et al.* (2000). The hatching index, number of nauplius and copepodids in each treatment were presented in the average percentage of 30 ovigerous females as in Equation 2.

$$\text{Hatching index} = \frac{\begin{array}{c}\text{(Number of eggs introduced initially}\\ \text{- Number of unhatched eggs) x 100}\end{array}}{\text{Number of eggs introduced initially}} \quad (2)$$

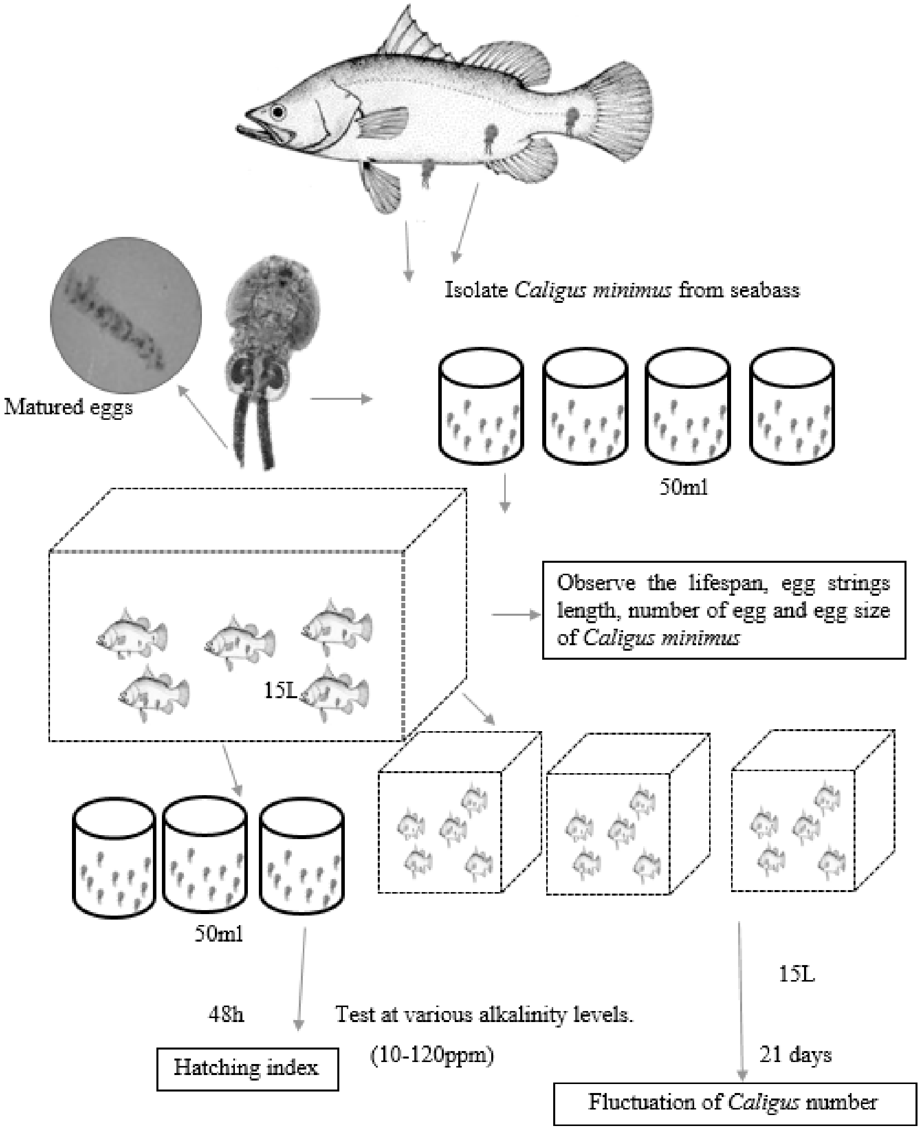

Figure 1: Diagram of the experimental set.

Parasite survivability at various alkalinity levels

A total of 10 ovigerous *C. minimus* parasites with egg strings were introduced to five seabass fingerling hosts weighing 10 ± 2 g in 15 L transparent aquariums. The aquariums were maintained in triplicates at room temperature, with 25 ppt of salinity and low aeration for 21 days. There were 12 levels of alkalinity from 10 to 120 ppm. Each level was applied for 48 hours and $NaHCO_3$ was used to increase alkalinity while maintaining the pH at 7.8. The fish were fed daily with a controlled amount of commercial pellets and the parasites' egg strings were observed.

Water was changed every three days via a drained pipe installed with a plankton filter (25 µm). At the end of the experiment, all the hosts were killed and the *parasites* collected and examined under a dissecting microscope.

Statistical analysis

Data were analyzed using IBM SPSS software Version 24.0 (IBM, Armonk, New York, USA) and Origin 2018 (OriginLab, Northampton, Massachusetts, USA). One-way ANOVA was used to determine the significant variations ($p < 0.05$) between fecundity and fluctuation of *Caligus* presence at different alkalinity levels. Significant differences in treatments were further subjected to Duncan's post-hoc test.

Results and Discussion

Fecundity under different alkalinity levels

Throughout the experiment, the water used to transport the fish and parasites was kept between 25.5 and 31 ºC, at a pH of 7.8, 25 to 30.5 ppt of salinity and 50 to 70 ppm of alkalinity.

The length of egg strings from 100 females was observed to be 3.38 ± 0.27 mm, and each brood was carrying 60.9 ± 14.3 eggs in its string. The egg string length was shortened after matured eggs were released. Matured eggs normally ruptured from the distal sac. However, the hatching caused the breaking of egg strings at un-sequenced parts (Figure 2).

Compared with other studies in Table 1, the egg string length and number of eggs in *C. minimus* were higher than *C .rogercresseyi, Lepeophtheirus pectoralis* and other freshwater copepods. However, the egg size of this parasite was smaller. This reproductive characteristics gave *C. minimus* the ability to quickly multiply and cause damage in a fish population within a short time.

Table 1: Fecundity of *Caligus minimus* and some crustacean parasites.

Species	Egg string length (mm)	Number of egg/female	Egg size (mm)	Host	Source
Caligus rogercresseyi	-	45 ± 16	-	*Salmo salar*	Bravo *et al.* (2009)
Caligus rogercresseyi	1.5-3.1	12-56	-	*Eleginops maclovinus*	GonzaŁlez *et al.* (2012)
Caligus rogercresseyi	4.9-6.1	19-95	-	*Oncorhynchus mykiss*	GonzaŁlez *et al.* (2012)
Caligus rogercresseyi	5.07	59 ± 19	-	*Salmo salar*	Bravo *et al.* (2013)
Lepeophtheirus salmonis	-	1000	-	-	Costello (1993)
Calanidae sp	-	40	0.152	-	Poulin (1995)
Pseudodiaptomidae sp	-	23	0.075	-	Poulin (1995)
Diaptomidae sp	-	29	0.086	-	Poulin (1995)
Porcellidiidae sp	-	11	0.085	-	Poulin (1995)
Lepeophtheirus pectoralis	3.36	45.5 ± 18.7	0.077	*Platichthys flesu*	Frade *et al.*, (2015)
Lepeophtheirus salmonis	8-40	50-500		*Salmo trutta*	Tully & Whelan (1993)

The ovigerous *C. minimus* survived up to three or four days without a host under laboratory conditions. Adult *C. minimus* females produced one to three egg strings in its life. The egg size was 0.34 mm wide and 0.08 mm long. The copepod lifecycle began as nauplius larvaes, which metamorphosized into copepodids, chalimi, pre-adult and adults through molting.

The parasites started to attach to the host from the copepodid stage. Through observation of 100 specimens, the lifespan of *Caligus minimus* was between 50 and 71 days on their host (61.5±10.5 days) (Figure 2).

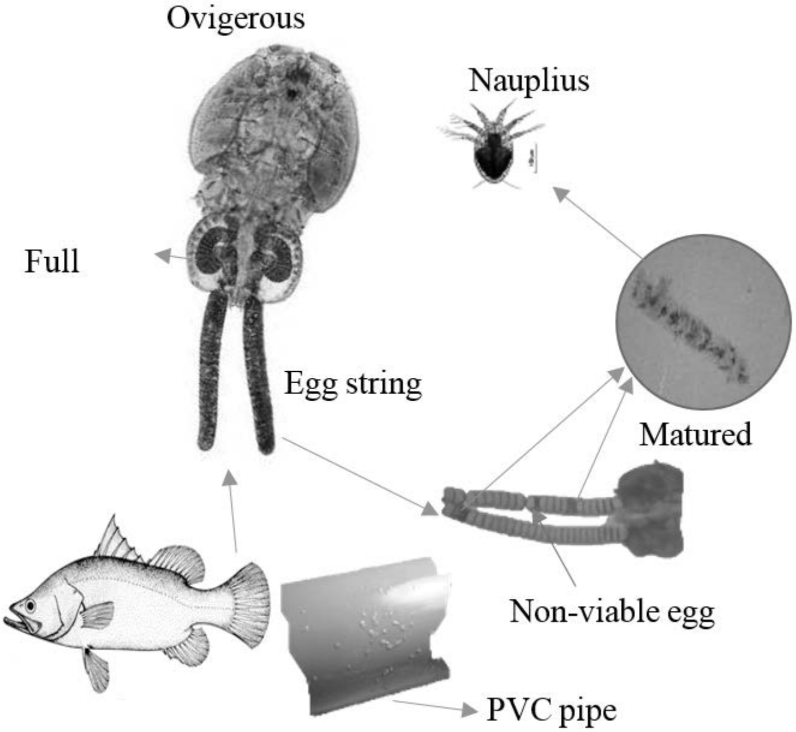

Figure 2: *C. minimus* could survive with a fish host/or without host by attaching on temporary substrate.

Based on initial egg string length and egg size of *Caligus*, the number of eggs and percentage of viable and non-viable eggs were not significantly different between aquariums. The alkalinity levels did not significantly affect egg size and number.

The interaction between parasites and host in an ecosystem had been studied to formulate strategies to control infestations. Several studies were conducted on *C. rogercresseyi* (Bravo *et al.,* 2009; GonzaŁlez *et al.,* 2012; Sandra Bravo *et al.,* 2013), *L. simplex* (Serna *et al.,* 2015), *L. salmonis* (Tully & Whelan, 1993; Ugelvik *et al.,* 2017) and *L. pectoralis* (Frade *et al.,* 2015). However, these studies were conducted in temperate zones and there were very few studies in tropical waters.

Heuch *et al.* (2000) noted that a higher water temperature positively affected the copepods' egg production and development, whereby greater fecundity and shorter life cycle were observed in *L. salmonis*. When cultured at 8.7 ºC, the parasites' egg strings were longer by seven per cent than their counterparts at 12.2 ºC. However, it took approximately four times longer for *L. salmonis* to produce an egg string at 7.2 °C than 12.2 ºC (Heuch *et al.,* 2000).

This study showed a great fecundity of *C. minimus* in warm waters. The ovigerous female carried 50 to 70 eggs on a pair of egg strings, similar to *C. rogercresseyi* found parasitizing on *S. salar* (Sandra Bravo *et al.,* 2013). But that figure was far lower compared to *L. salmonis* and *L. pectoralis*, which had up to 500 to 1000 eggs in each brood.

The fecundity difference could be influenced by many environmental factors, such as temperature (Sarvala, 1979; Tully, 1989; Heuch *et al.,* 2000), female body size (Tully & Whelan, 1993), type of host (farmed or wild type) (Tully & Whelan, 1993; Nordhagen & Schram, 2000) and salinity level (Muhd-Faizul *et al.,* 2012). Therefore, the significant difference in water temperature between temperate and tropical zones could lead to greater egg production and metamorphosis of copepods in warm conditions.

The life cycle of *C. rogercresseyi* could be prolonged from 31 to 45 days in waters ranging from 10.3 to 12.8 ºC. *C. elongates* could survive for 43.3 days at 10 ºC. In warmer temperatures, C. pageti could only live for 10 to 11 days at 26 ºC (Russell, 1925), while the lifespan of *C. minimus* was even shorter at five to seven days at 29.5 ºC (Khoa, 2015; Khoa *et al.,* 2018). Additionally, *L. latis* took just 222 hours to complete its life cycle at 30 ºC (Brazenor & Hutson, 2013; Qamarina *et al.,* 2014). This explained the challenges posed by crustacean parasites in tropical waters due to rapid multiplication.

Hatching index (%)

A higher alkalinity level could negatively affect the hatching index of copepods. The highest rate of hatching was observed between 60 ppm (81.99%) and 70 ppm (85.27%) of alkalinity levels (Figure 3), which was significantly different compared to other levels of treatment. The eggs were observed to take up water through osmosis, enlarging their internal membranes, but no hatching was recorded at 10, 20, 110 and 120 ppm of alkalinity. The result also showed a limited hatching of eggs at 30 ppm and 100 ppm of alkalinity. Plot analysis suggested that hatching index could peak at 65 ppm, and the approximate range of alkalinity for *C. minimus* to hatch was from 45 ppm to 80 ppm, where the hatching index was above 50%.

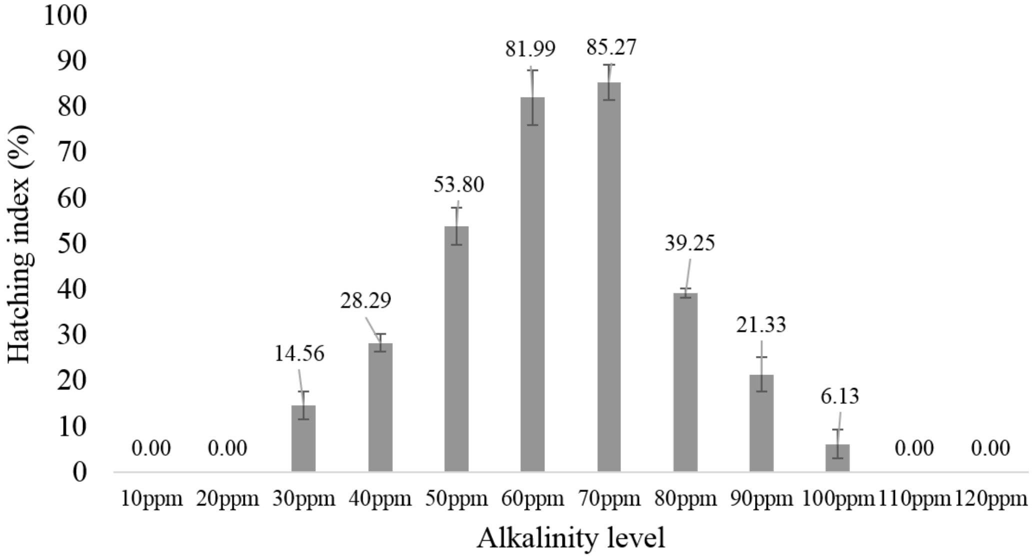

Figure 3: Hatching index of *Caligus minimus* egg in various alkalinity levels.

The presence of *C. minimus* strongly fluctuated at different alkalinity levels (Figure 4). The highest infection was recorded at 60 and 70 ppm (734 ± 14.5% and 919 ± 25.5% of the initial infection, respectively), and were significantly different compared to other levels. The high mortality rate of *C. minimus* in 10, 20, 30, 110, 120 ppm of alkalinity caused a reduction in parasite population, and none of the organism was observed on the fish at the end of the experiment. At between 40 and 100 ppm, the parasite population slightly increased while a higher population dynamics was observed in 50, 80 and 90 ppm of alkalinity. However, there were no significant differences observed among these treatments.

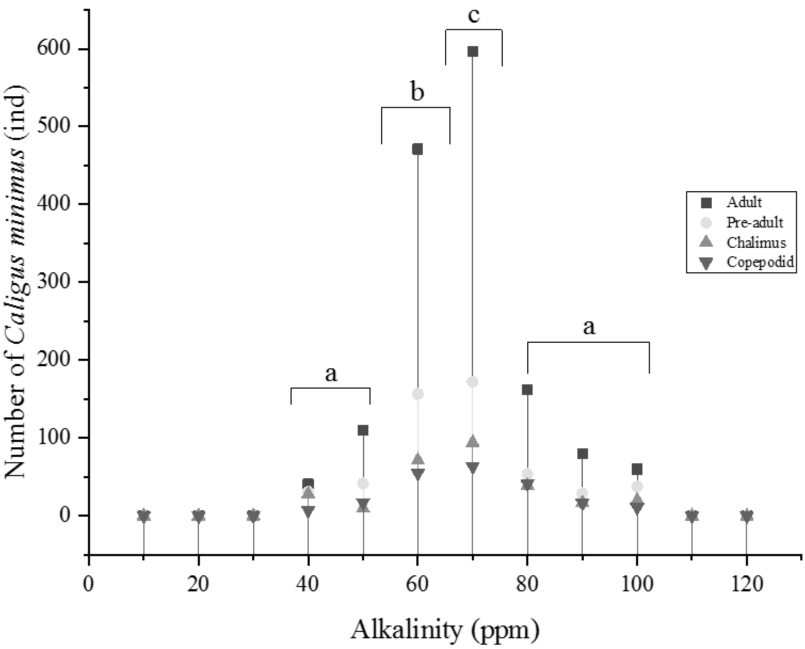

Figure 4: Population dynamic of *Caligus minimus* in various alkalinity levels.

In the marine ecosystem, pH is an important factor that affects the viability of marine life (Doney *et al,* 2009; Omstedt *et al.,* 2010), and is characterized by total alkalinity (Omstedt *et al.,* 2010). The rise in water alkalinity could negatively affect copepod populations (Garzke *et al.,* 2016) and this imbalance would lead to disorder in the organisms' metabolism (Pörtner *et al.,* 2004; Michaelidis *et al.,* 2005). Metabolic depression had been suggested to affect copepod fecundity (Mayor *et al.,* 2007). In egg development and hatching, a decrease in pH and alkalinity would interfere in egg membrane depolarization during fertilization (McCulloh *et al.,* 1987), depress respiration and protein synthesis in gametes and embryos (Spikes, 1983), and cause genetic abnormalities in egg cells (Pagano *et al.,* 1985; Stack *et al.,* 2006). Hence, egg mortality dramatically affects the hatching index and abundance of copepod parasites.

Female copepods would suffer from low egg production (Vehmaa *et al.,* 2012; Zervoudaki *et al.,* 2014) and produce a high number of unfertilized, non-viable or viable eggs in quiescent states (Cripps *et al.,* 2014). Mass mortality of parasites had also been observed (Pedersen *et al.,* 2013) as well as impairment of the crustacean's post-moulting stage (Borisov *et al.,* 2007; Michaelidis *et al.,* 2005). This study was limited by its short duration (21 days) and it could not evaluate the effect of alkalinity on egg number and size.

Conclusion

The current study evaluated the fecundity of *C. minimus* and the effect of alkalinity on the parasite's fertility. The optimal alkalinity levels for copepods to thrive on seabass hosts was between 60 and 70 ppm. However, further studies need to investigate the population dynamics of *C. minimus* under various environmental factors to model epidemiological progress and develop effective management strategies for commercial aquaculture.

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