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Diel variations in *Centropages tenuiremis* (Copepoda) feeding, spawning and its relationship with temperature

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Diel rhythms in feeding and spawning were investigated in Centropages tenuiremis *from Xiamen Bay in March to May, 2006. Circular statistics were used to determine the peak time of spawning. The results showed that the feeding activities of females were stably higher at night-time, and there was a remarkable earlier shift in spawning peak time with warmer seawater. Thus, the lag times between peak times of gut pigment content and spawning were shortened with the increase of temperature. It suggested that there was a direct effect of feeding rhythms on egg production variations in copepods, and the seawater temperature would work on the converting time and then influence the spawning peak time. So the effect of temperature cannot be ignored in the investigation of the effects of feeding on egg production.*

Keywords: feeding, spawning, diel, temperature, circular distribution

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INTRODUCTION

Diel rhythms of feeding have been reported for several calanoid copepods including *Centropages furcatus* (Checkley *et al.*, 1992), *C. typicus* (Dagg & Grill, 1980; Christaki *et al.*, 1998; Calbet *et al.*, 1999, 2007), *Acartia clausi* (Kouassi *et al.*, 2001; Pagano *et al.*, 2004), *A. longiremis*, *A. omorii* (Saito & Taguchi, 1996), *A. tonsa* (Hassett & Blades-Eckelbarger, 1995), *Calanus euxinus* (Besiktepe *et al.*, 2005), *C. finmarchicus* (Marshall & Orr, 1955) and *C. pacificus* (Dagg *et al.*, 1998) with ingestion mostly at night. Many copepod species also exhibit a diel rhythm in egg production: e.g. *Acartia tonsa* (Stearns *et al.*, 1989; Cervetto *et al.*, 1993), *C. pacificus* (Runge, 1985), *C. finmarchicus* (Harding *et al.*, 1951), *C. helgolandicus* (Mullin, 1968), *Labidocera aestiva* (Marcus, 1985), *A. erythraea*, *A. pacifica* and *C. furcatus* (Checkley *et al.*, 1992). Gophen (1978) suggested that the diel egg production of copepods was a reflection of rhythmic feeding. Many studies have considered the combinations of these two processes, feeding and spawning, but most of those were restricted to the quantitative relationship between them, e.g. *C. finmarchicus* (Mayor *et al.*, 2006) and *C. furcatus* (Checkley *et al.*, 1992). Only a few studies have examined the lag times between maxima of feeding and spawning rates of several species (Stearns *et al.*, 1989; Tester & Turner, 1990; Cervetto *et al.*, 1993; Pagano *et al.*, 2004). And there is considerable interspecific variability in the lag time for conversion of ingested food to egg production (Tester & Turner, 1990). Yet other factors associated with the lag time have not been demonstrated. Wu *et al.* (2006) had assumed that the seawater temperature would work on the lag times of copepods, since it is one of the most important environmental factors to influence the metabolic velocity of animals; to date, this has not been proven.

Centropages tenuiremis is a common and dominant copepod species in Xiamen Bay (Huang *et al.*, 1986; Wu *et al.*, 2007), and especially in winter–spring it plays a prominent role in terms of biomass in the zooplankton community. The aim of this study was to investigate the feeding and spawning diel rhythms of *C. tenuiremis* in relation to temperatures in Xiamen Bay, in order to analyse their relationships and to examine the effect of seawater temperature on the lag times between their peak times.

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MATERIALS AND METHODS

Diel variations in gut pigment content and egg production of *Centropages tenuiremis* in Xiamen Bay, People's Republic of China were measured in this study. Five experiments were carried out from March to May 2006.

Sampling

Zooplankton samples were collected at night using a plankton net with 300 μm mesh on the surface of Xiamen waters, People's Republic of China. The samples were carefully diluted into 15 l polythene barrels with ambient seawater and transported to the laboratory within 30 minutes. The ambient seawater (~40 l) for incubation was collected through 50 μm filters, and its temperature was measured at the same time. In the laboratory within about 30 minutes, the vigorous and mature females of *C. tenuiremis* were selected using a wide-mouth pipette for spawning and feeding experiments.

Feeding experiments

Diel variation in feeding was determined by measuring the gut pigment contents of pooled samples of copepods over a 24 hour period. The selected females (~300 ind.) were bulk incubated in two incubators containing approximately 2 l of fresh seawater (i.e. *in situ* seawater filtered through 50 μm mesh), and the light condition was natural light in laboratory without direct radiation. One sample of 20 females was gently sorted out from each of the incubators at 4 hour intervals and immediately filtered onto a 100 μm nylon sieve and rinsed with 0.45 μm filtered seawater. The sample was transferred to a 5 ml centrifuge tube and then kept in the dark at −20°C. 3 ml 90% acetone solution was added in the tube for pigment extraction during 12 hours at 4°C in darkness. Fluorescence was then measured with a Shimadzu RF25301 fluorometer before and after acidification. Chlorophyll-*a*-equivalent gut content in zooplankton was estimated following the methods of Parsons *et al.* (1984) and used as an index of feeding activity.

Spawning experiments

The selected females were individually introduced into incubators containing approximately 40 ml fresh seawater. To avoid the bias of egg production *in situ* from experimental food (Saiz *et al.*, 1997), the ambient seawater filtered with 50 μm mesh from sampling site was used as food source during experimental incubation. The incubators were modified from Burkart & Kleppel (1998) under natural light conditions in the laboratory without direct radiation. Samples of 24 females were tested at 4 hour intervals over 24 hours, and at the same time the incubating seawater was renewed. During these examinations the numbers of eggs produced by each female were counted under a dissecting microscope. The individual daily egg production rate was calculated by integrating these egg productions over a 24 hour period.

Since clutch size and egg production of different females in a species, even the same female in different days, varies depending on female size and other factors (Runge, 1984), relative production rates of night-time and daytime were used to examine the seasonal variations in diel production pattern.

Statistical analysis

One-way analysis of variance (ANOVA) was conducted to compare the seasonal differences in egg production rates. Cochran and Kolmogorov–Smirnov tests were used to test for the normality and homogeneity of variances, respectively, before the comparisons. To avoid the abnormal distribution resulted from much zero value data in each examination, only females spawned were analysed. Statistical analyses were performed using the SPSS 16.0 statistical package.

Diel variations in egg production of *C. tenuiremis* were analysed by circular statistics (Zar, 2010). The median time of each examination interval was transformed into an angular direction, and the histogram bar represents the percentage of eggs laid during this interval in one day. The peak time and the intensity of egg production were evaluated by calculation of a mean vector defined by a mean angle (α) and a mean length (*r*). The significance of the length of the mean vector was tested with the Rayleigh test (*z*). Circular distribution plots were graphed using Grapher 8.2.460 software showing the diel changes in the eggs production of *C. tenuiremis*.

RESULTS

Gut pigment content

A nocturnal increase of the gut pigment of *Centropages tenuiremis* females was observed from March to May (Figure 1). In general, the copepods had the highest gut contents during the night (about 3 a.m.), the lowest ones around noon, and the second peaks during the dusk (about 7 p.m). The only exception is on 18 March with a lack of examination at 3 a.m. A two-way ANOVA confirmed that this diel variation was significant when considering the 5 surveys together (for diel effect, $P = 0.022$; for date, $P = 0.224$). Slightly lower mean value of gut pigment content was found in May compared with March and April, but this was not significant (Table 1).

Spawning

Daily egg production rates of *C. tenuiremis* varied remarkably in the different surveys (Table 1, $F = 4.04$, $P = 0.005$). The

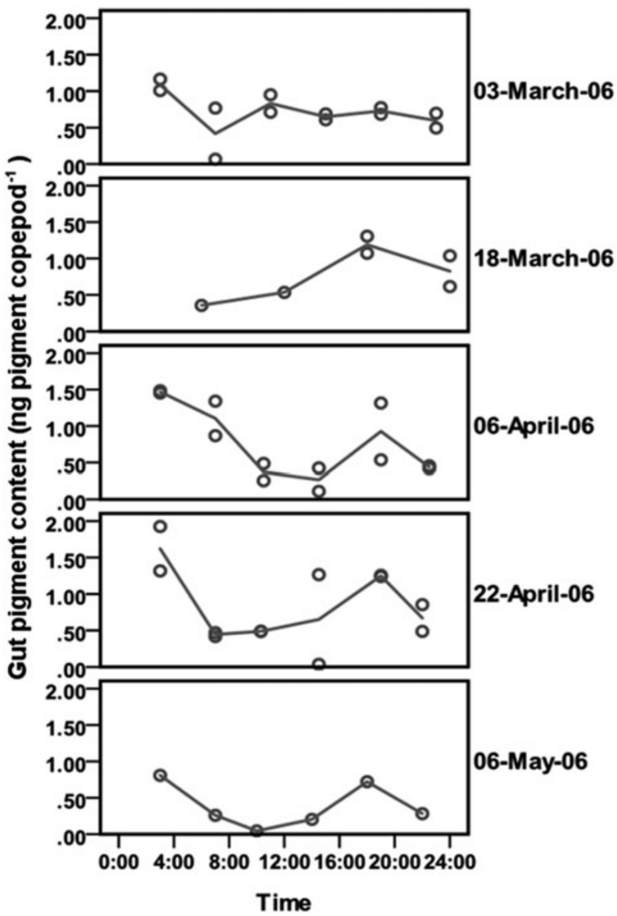

Fig. 1. Daily variations of gut pigment contents of copepods (circles) and of average values (continuous lines).

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Table 1. Date, temperature, egg production rate, and gut pigment content.

Date	Temperature °C	Egg production duration 24 hours eggs female^{-1} d^{-1} (means ± SD)	Gut pigment content ng pigment ind^{-1} (means ± SD)
3 March 2006	15	14.3 ± 16.9, N = 24 (16)	0.683 ± 0.223
18 March 2006	16	28.8 ± 35.9, N = 24 (17)	0.728 ± 0.364
6 April 2006	18	34.5 ± 31.2, N = 24 (18)	0.763 ± 0.478
22 April 2006	20.5	14.6 ± 20.8, N = 24 (17)	0.854 ± 0.474
6 May 2006	22.5	16.7 ± 17.8, N = 24 (18)	0.389 ± 0.305

SD, standard deviation; the numbers of spawning females are shown in parentheses.

present results showed that egg production rates were higher during the cold periods than the warm periods. And there was no significant correlation between mean daily feeding activity and egg production rate ($P = 0.692$, N = 5).

Diel variation in egg production rate was apparent, but the patterns were different in each survey (Figure 2). The spawning peak mainly occurred earlier with the increasing of temperature. The relative rates were much higher during night than day on 3 March (paired t-test, $t = 3.22$, $P = 0.003$; time classification by grouping day and night value) and 18 ($t = 1.76$, $P = 0.049$), while it was not the case on 6 April ($t = -0.178$, $P = 0.431$) and 22 ($t = -0.708$, $P = 0.245$). However, the relative rates were much lower during night than day on 6 May ($t = -2.08$, $P = 0.025$).

Lag time

The lag times between peak times of gut pigment content and spawning could be estimated from the data above. A negative linear relationship between time and temperature was observed (Figure 3; time = −1.265 temperature + 38.533, $r = 0.912$, $P = 0.031$). The lag time in March was long, whereas in May it was short.

DISCUSSION

Diel rhythms in feeding and spawning were investigated in *Centropages tenuiremis* from Xiamen waters. Their feeding activities were higher during the night-time and their spawning peak occurred earlier during warmer seasons.

Feeding rhythms

The gut pigment content could reflect the feeding activity of copepods, although there was some bias for omnivorous copepods (Boyd *et al.*, 1980). High feeding activity during the night was confirmed for *C. tenuiremis* in the incubation experiment from 3 March to 6 May 2006. Nocturnal feeding may be a common behaviour in planktonic copepods, having been documented for many species, and especially for *C. typicus* even under 'low' natural food (Calbet *et al.*, 1999). Several hypotheses for diel feeding mechanism had been proposed: e.g. diel vertical migration (Boyd *et al.*, 1980), diel changes in food availability (Huntley & Brooks, 1982), escape of predation (Stearns, 1986), endogenous feeding rhythm (Stearns, 1986; Calbet *et al.*, 1999), etc. The nocturnal feeding pattern of *C. tenuiremis* was not obviously originated by diel changes in food availability, since the food source was the same, i.e. 50 μm filtered seawater *in situ* collected the day before. We speculated that the persistence of a diel feeding rhythm in *C. tenuiremis* was indicative of an endogenous feeding rhythm. This feeding pattern might result from

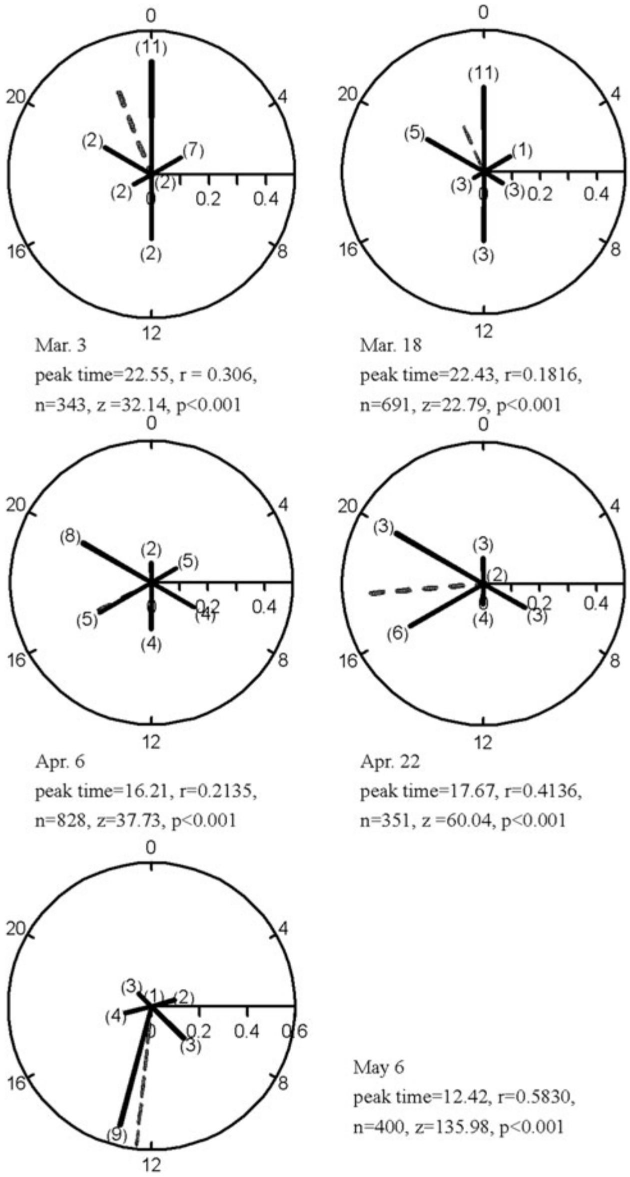

Fig. 2. Circular distribution showing the diel changes in the eggs production of *Centropages tenuiremis*. The numbers of spawning females are shown in parentheses. The direction of the broken line indicates the mean angle (i.e. the peak time), and the length of the line expressed r (i.e. the intensity of egg production). n, the number of eggs produced; z, the value of Rayleigh test for circular distribution.

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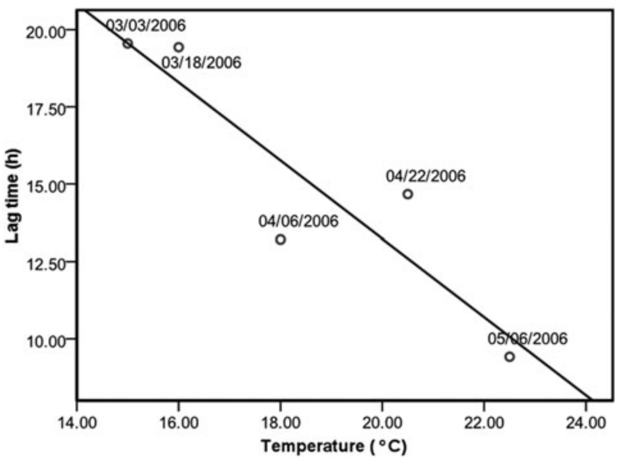

Fig. 3. Relationship between lag time and temperature. Lag time = −1.265 temperature + 38.533 ($r = 0.912$, $P = 0.031$). Lag time represents the time interval between peak times of gut pigment content and spawning.

previous feeding history (Huntley, 1988) and be formed by cyclical pattern in gut-cell development (Hassett & Blades-Eckelbarger, 1995). Perhaps the endogenous nocturnal feeding pattern was the response to *in situ* diel vertical migration (Wu *et al.,* 2006). It was not certain whether there was a food limit influencing the feeding rhythms in Xiamen waters during the experimental period, for the lack of measuring the chlorophyll-*a* concentration *in situ*. But in general scarcely was the concentration of phytoplankton very low during March to May in Xiamen coastal waters (Wu *et al.,* 2007). However, the diel feeding pattern of *C. tenuiremis* was stable whatever the food composition and quantity according to the consistent feeding rhythms in March to May.

Spawning rhythms and lag time

Reproduction is an important process in population dynamics. Many studies, both experimental and field, have shown that egg production depends on both maternal factors (e.g. size of females and gonad stage) and environmental conditions (e.g. available food and temperature). Some investigations have suggested that there was a direct effect of feeding rhythms on spawning variations (Stearns *et al.*, 1989; Tester & Turner, 1990; Cervetto *et al.*, 1993; Wu *et al.*, 2006). Our results showed that the peak times for spawning varied with temperature *in situ*, that is to say, the lag times between peak times of gut pigment content and spawning were different during the experimental period.

There was considerable interspecific variability in the time required for copepods to convert ingested food to egg production (Tester & Turner, 1990). And the lag times for females often revealed their different gonad morphology and maturation types. For example, the lag time for *Acartia tonsa* (9.5 hours) with *Acartia*-type gonad was much shorter than for *C. typicus* (89 hours) with *Calaus*-type gonad in similar cold season (Tester & Turner, 1990; Niehoff, 2007). Unlike *Calanus* species, *Centropages* species apparently have no internal store that can fuel oocyte development and the release of eggs (Marshall & Orr, 1952; Niehoff, 2007). Thus, *Centropages* species were more vulnerable to direct effect of feeding behaviour than *Calanus* species.

Hirche *et al.* (1997) found that the response time of egg production to feeding was related to temperature. The effect of temperature on lag times is not surprising since most metabolic processes (such as the rate of gut evacuation) depend on temperature (Christoffersen & Jespersen, 1986). Our results showed that the spawning peak times for *C. tenuiremis* were earlier in warmer periods, just as in *Calanus finmarchicus* (Marshall & Orr, 1952). So we confirmed our suggestion that the warmer seawater temperature *in situ* accelerated the process of converting ingested food to egg production in copepods (Wu *et al.*, 2006). Thus, the effect of temperature cannot be ignored in the investigation of the population dynamics of this copepod. For example, when seawater temperatures rise quickly, the daily fecundity is likely to be over-estimated in experiments without regard to the shortened lag time, and vice versa. However, temperature is not the only factor affecting the lag times. The nutrient value of food ingested could also impact on them, since high value food is more effective for egg production (Marshall & Orr, 1961).

In summary, a stable nocturnal feeding pattern and a remarkable earlier shift in spawning peak time with warmer seawater were revealed in this study. There was a direct effect of feeding rhythms on egg production variations in copepods, and the seawater temperature would work on the converting time (from ingested food to egg production) and then influence the spawning peak time. So the effect of temperature cannot be ignored in the investigation of the effects of feeding on egg production.

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REFERENCES

Besiktepe S., Svetlichny L., Yuneva T., Romanova and Shulman G. (2005) Diurnal gut pigment rhythm and metabolic rate of *Calanus euxinus* in the Black Sea. *Marine Biology* 146, 1189–1198.

Boyd C.M., Smith S.L. and Cowles T.J. (1980) Grazing patterns of copepods in the upwelling system off Peru. *Limnology and Oceanography* 25, 583–596.

Burkart C.A. and Kleppel G.S. (1998) A new incubation system for the measurement of copepod egg production and egg hatching success in the field. *Journal of Experimental Marine Biology and Ecology* 221, 89–97.

Calbet A., Saiz E., Irigoien X., Alcaraz M. and Trepat J. (1999) Food availability and diel feeding rhythms in the marine copepods *Acartia grani* and *Centropages typicus. Journal of Plankton Research* 21, 1009–1015.

Calbet A., Carlotti F. and Gaudy R. (2007) The feeding ecology of the copepod *Centropages typicus* (Kröyer). *Progress in Oceanography* 72, 137–150.

Cervetto G., Gaudy R., Pagano M., Saint-Jean L., Verriopoulos G., Arfi R. and Leveau M. (1993) Diel variations in *Acartia tonsa* feeding, respiration and egg production in a Mediterranean coastal lagoon. *Journal of Plankton Research* 15, 1207–1228.

Checkley D.M., Dagg M.J. and Uye S. (1992) Feeding, excretion and egg production of the marine planktonic copepods *Acartia* spp. and *Centropages furcatus. Journal of Plankton Research* 14, 71–96.

https://doi.org/10.1017/S0025315412001026 Published online by Cambridge University Press

Christaki U., Gaudy R. and Kerambrum P. (1998) A study of the effects of migratory zooplankton on microbial populations in surface waters of the N.W. Mediterranean. *Journal of Experimental Marine Biology and Ecology* 225, 173–183.

Christoffersen K. and Jespersen A.M. (1986) Gut evacuation rates of *Eudiaptomus graciloides* measured by means of the gut fluorescence method. *Journal of Plankton Research* 8, 973–983.

Dagg M.J. and Grill D.W. (1980) Natural feeding rates of *Centropages typicus* females in the New York Bight. *Limnology and Oceanography* 25, 597–609.

Dagg M.J., Frost B.W. and Newton J. (1998) Diel vertical migration and feeding in adult female *Calanus pacificus*, *Metridia lucens* and *Pseudocalanus newmani* during a spring bloom in Dabob Bay, a fjord in Washington USA. *Journal of Marine Systems* 15, 503–509.

Gophen M. (1978) Errors in the estimation of recruitment of early stages of *Mesocyclops leuckarti* (Claus) caused by the diurnal periodicity of egg production. *Hydrobiologia* 57, 59–64.

Harding J.P., Marshall S.M. and Orr A.P. (1951) Time of egg-laying in the planktonic copepod *Calanus*. *Nature* 167, 953.

Hassett R.P. and Blades-Eckelbarger P. (1995) Diel changes in gut-cell morphology and digestive activity of the marine copepod *Acartia tonsa*. *Marine Biology* 124, 59–69.

Hirche H.J., Meyer U. and Nieho B. (1997) Egg production of *Calanus finmarchicus*: effect of temperature, food and season. *Marine Biology* 127, 609–620.

Huang J.Q., Xu Z.Z. and Li Y.R. (1986) Preliminary studies on the diel vertical removal of planktonic Crustacea in Xiamen Harbour. *Acta Oceanologica Sinica* 8, 215–222. [In Chinese.]

Huntley M.E. and Brooks E.R. (1982) Effects of age and food availability on diel vertical migration of *Calanus pacificus*. *Marine Biology* 71, 23–31.

Huntley M.E. (1988) Feeding biology of *Calanus*: a new perspective. *Hydrobiologia* 167/168, 83–99.

Kouassi E., Pagano M., Saint-Jean L., Arfi R. and Bouvy M. (2001) Vertical migrations and feeding rhythms of *Acartia clausi* and *Pseudodiaptomus hessei* (Copepoda: Calanoida) in a tropical lagoon (Ebrie, Côte d'Ivoire). *Estuarine, Coastal and Shelf Science* 52, 715–728.

Marcus N.H. (1985) Endogenous control of spawning in a marine copepod. *Journal of Experimental Marine Biology and Ecology* 91, 263–269.

Marshall S.M. and Orr A.P. (1952) On the biology of *Calanus finmarchicus*. VII. Factors affecting egg production. *Journal of the Marine Biological Association of the United Kingdom* 30, 527–549.

Marshall S.M. and Orr A.P. (1955) Experimental feeding of the copepod *Calanus finmarchicus* (Gunner) on phytoplankton cultures labelled with radioactive carbon (^{14}C). *Deep-Sea Research* 3, 110–114.

Marshall S.M. and Orr A.P. (1961) On the biology of *Calanus finmarchicus*. XII. The phosphorus cycle: excretion, egg production, autolysis. *Journal of the Marine Biological Association of the United Kingdom* 41, 463–488.

Mayor D.J., Anderson T.R., Irigoien X. and Harris R. (2006) Feeding and reproduction of *Calanus finmarchicus* during non-bloom conditions in the Irminger Sea. *Journal of Plankton Research* 28, 1167–1179.

Mullin C.H. (1968) Egg-laying in the planktonic copepod *Calanus helgolandicus*. *Crustaceana* (Supplement) 1, 29–34.

Niehoff B. (2007) Life history strategies in zooplankton communities: the significance of female gonad morphology and maturation types for the reproductive biology of marine calanoid copepods. *Progress in Oceanography* 74, 1–47.

Pagano M., Kouassi E., Arfi R., Bouvy M. and Saint-Jean L. (2004) *In situ* spawning rate of the calanoid copepod *Acartia clausi* in a tropical lagoon (Ebrie, Côte d'lvoire): diel variations and effects of environmental factors. *Zoological Studies* 43, 244–254.

Parsons T.R., Maita Y. and Lalli G.M. (eds) (1984) *A manual of chemical and biological methods for seawater analysis*. Oxford: Pergamon Press.

Runge J.A. (1984) Egg production of the marine, planktonic copepod, *Calanus pacificus* Brodsky: laboratory observations. *Journal of Experimental Marine Biology and Ecology* 74, 53–66.

Runge J.A. (1985) Relationship of egg production of *Calanus pacificus* to seasonal changes in phytoplankton availability in Puget Sound, Washington. *Limnology and Oceanography* 30, 382–396.

Saito H. and Taguchi S. (1996) Diel feeding behavior of neritic copepods during spring and fall blooms in Akkeshi Bay, eastern coast of Hokkaido, Japan. *Marine Biology* 125, 97–107.

Saiz E., Calbet A., Trepat I., Irigoien X. and Alcaraz M. (1997) Food availability as a potential source of bias in the egg production method for copepods. *Journal of Plankton Research* 19, 1–14.

Stearns D.E. (1986) Copepod grazing behaviour in simulated natural light and its relation to nocturnal feeding. *Marine Ecology Progress Series* 30, 65–76.

Stearns D.E., Tester P. and Walker R.L. (1989) Diel changes in the egg production rate of *Acartia tonsa* (Copepoda, Calanoida) and relation to environmental factors in two estuaries. *Marine Ecology Progress Series* 52, 7–16.

Tester P.A. and Turner J.T. (1990) How long does it take copepods to make eggs? *Journal of Experimental Marine Biology and Ecology* 141, 169–182.

Wu L., Wang G. and Li S. (2006) Seasonal variation in diel production pattern of *Centropages tenuiremis* in Xiamen waters, China. *Hydrobiologia* 559, 225–231.

Wu L., Wang G., Jiang X. and Li S.J. (2007) Seasonal reproductive biology of *Centropages tenuiremis* (Copepoda) in Xiamen waters, People's Republic of China. *Journal of Plankton Research* 29, 437–446.

and

Zar J.H. (2010) *Biostatistical analysis*. 5th edition. Upper Saddle River, NJ: Prentice-Hall.

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