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DORMANT EGGS OF A CALANOID COPEPOD FROM TROPICAL BRACKISH AQUACULTURE PONDS

BY

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ABSTRACT

Many Asian aquaculture farms cultivate copepods as live-feed for aquaculture species, as copepod nutritional and behavioural qualities are usually higher than those of traditional live-feeds (i.e., *Artemia* and rotifers). The most convenient method to use zooplankton in aquaculture as live feed involves dormant eggs. In this study, to establish whether the copepod species of the tropical Taiwanese ponds exhibit quiescence of their eggs, several sediment samples were collected from the ponds and incubated at low temperature. After 3 months of cold incubation, several nauplii of *Acartia bilobata* Abraham, 1970 hatched from the sediment at high temperature over several days. Further investigations need to be conducted to understand the origin of the dormancy (i.e., induced originally in the field or in the laboratory). Nevertheless, it is the first reported case of dormant eggs in calanoid copepods in tropical regions. The present study will certainly find some interest in tropical aquaculture as our results showed that long-term storage of tropical calanoid copepod eggs is possible, even if collected directly from the sediment without sorting the eggs.

RÉSUMÉ

Plusieurs fermes aquacoles en Asie cultivent des copépodes servants de nourriture vivante aux espèces piscicoles car leurs qualités nutritionnelles et comportementales sont généralement meilleures que celles des nourritures traditionnelles (i.e. artémia et rotifères). La méthode la plus adaptée pour utiliser du zooplancton vivant en aquaculture implique des oeufs de dormance. Dans cette étude, afin de déterminer si les copépodes issus des étangs tropicaux d'aquaculture de Taïwan produisent des œufs de dormance, plusieurs échantillons de sédiments ont été prélevés dans les étangs puis incubés à basse température. Après 3 mois d'incubation à froid, plusieurs nauplii d'*Acartia bilobata* Abraham, 1970 ont éclos du sédiment sur plusieurs jours à température élevée. Des études complémentaires sont nécessaires afin de déterminer l'origine de la dormance observée

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(i.e., induite *in situ* ou en laboratoire). Néanmoins, c'est la première fois qu'un état d'oeuf de dormance est reporté chez un copépode calanoïde d'une région tropicale. Cette étude intéressera certainement l'aquaculture en milieu tropical car nos résultats montrent que la conservation à long terme d'oeufs de copépode calanoïde est possible même en étant directement conservé avec le sédiment sans isolation préalable des oeufs.

INTRODUCTION

The use of diapausing eggs or quiescent subitaneous eggs is pertinent in long-term storage of live-feed organisms in aquaculture (Drillet et al., 2006). The nutritional and behavioural qualities of calanoid copepods are generally assumed to be very good for marine fish larvae and of higher quality than those of commonly used live feeds (Shields et al., 1999; Støttrup, 2000; Evjemo et al., 2003). In the tropical regions of Taiwan, several outdoors aquaculture farms cultivate copepods in mass culture ponds to feed the larvae of their own commercial aquaculture species. The excesses of mature copepods are collected and sold to aquaculture farms or marine aquariums as frozen food to feed fish larvae, crustaceans, seahorses or corals (Woods, 2003).

In aquaculture of temperate regions, different methods for the production and storage of calanoid copepod eggs, mainly in resting stages, have been developed in recent years. Resting eggs can be used to initiate copepod culture stock and thus to freely dispose of live food. So far no references in the literature report dormant egg production in tropical calanoid copepods such as in Taiwan (Hwang & Martens, 2011) and the few attempts have failed (Schipp, 2006). Thus, the present study is a pilot research working on dormant eggs in tropical copepod ponds. The main objective of this experiment was therefore to establish whether the copepod species of the tropical Taiwanese ponds exhibit quiescence.

MATERIAL AND METHODS

In this study, sampling was conducted on 11 April 2007 in two different ponds of the same aquaculture farm (fig. 1). In the ponds, different species of rotifers and copepods, i.e., the cyclopoid *Apocyclops royi* (Lindberg, 1940) and the calanoids *Pseudodiaptomus annandalei* Sewell, 1919 and *Acartia bilobata* Abraham, 1970 are cultivated. The ponds that are on average 150 cm deep are used to culture the Pacific white shrimp (*Litopenaeus vannamei* (Boone, 1931)) that feed on zooplankton. The temperature range is 25-28°C in spring-summer and the salinity range is 20-22 g l^{-1}. The sediments were sampled in the ponds at 5 different locations on the side, in the middle and at three random locations in the ponds. Immediately after sampling the water above the sediment was removed (the cores

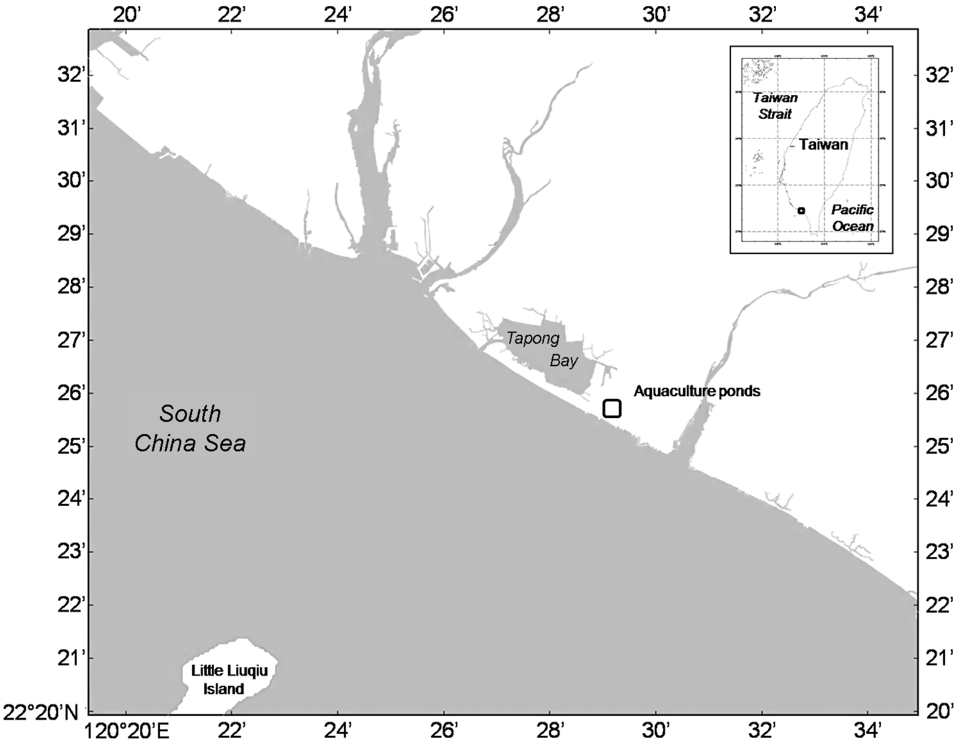

Fig. 1. Location of the aquaculture farm in southern Taiwan, near Tapong Bay.

were closed without air between the sediment surface and the cap) and stored in the dark. In the laboratory, each sediment sample was divided into 1-3 mm sub-samples and kept in Petri dishes. Photoperiod and temperature are among the most important factors that reactivate dormant the eggs (Alekseev, 2007); thus, we placed the Petri dishes in the dark at low temperature (4°C). For most of the eggs, the maintenance at low temperature for at least 3 months seems to be the most appropriate to complete the dormancy reactivation (Alekseev, 2007). Thus, 3 months later, 2 mm of brackish water at salinity 15 was added above the sediment and then the Petri dishes were placed into incubator chambers at 30°C under a 12L : 12D photoperiod. Once a day, the newly hatched nauplii were counted under a microscope and isolated from the Petri dishes. The water level in the Petri dish was maintained at 2 mm above the sediment. The observations were maintained for 2 weeks after the last hatching was observed. At any step, the eggs were sorted from the sediment. The nauplii hatched from the sediment were placed in a 1 litre beaker at salinity 15 and 30°C under mild aeration and daily fed *Isochrysis galbana* Parke, 1949. They were maintained under culture conditions until determination of the species at the adult stage.

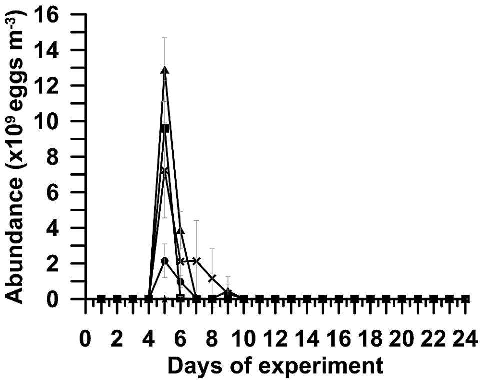

Fig. 2. Mean and standard error (errors bars) of nauplii hatched during 24 days of incubation at 30°C. Only results for pond B are presented since no hatching was observed in pond A. Sediment sampled on the side (circle), in the middle (triangle) and randomly (cross, square and star) of the ponds.

RESULTS

There was no hatching of copepod eggs from the sediment samples collected in pond A. In pond B, copepod nauplii hatched from 4 up to 5 sediment samples (fig. 2). The nauplii hatched from day 5 to day 9 after the beginning of the 30°C incubation. More than 60% of the nauplii hatched five days after incubation at 30°C (table I). The highest total abundance was found in the sediment sample from the middle of the pond with $(17.25 \pm 1.8) \times 10^9$ eggs m^{-3}. After determination, all the hatched eggs from the sediments appeared to belong to the copepod *Acartia bilobata* (Calanoida).

TABLE I

Proportion of nauplii ± standard deviation (%) hatched from the sediment after incubation at 30°C

Incubation time (days)	Sediment samples				
	I	II	III	IV	V
1-4	0	0	0	0	0
5	65.6 ± 19.0	74.6 ± 3.4	61.9 ± 35.6	0	99.3 ± 1.2
6	32.7 ± 16.8	22.3 ± 4.2	14.9 ± 13.0	0	0.7 ± 1.2
7	1.7 ± 2.2	0	14.26 ± 13.3	0	0
8	0	0.1 ± 0.1	7.2 ± 9.4	0	0
9	0	3.0 ± 5.3	1.8 ± 3.1	0	0
10-24	0	0	0	0	0

Sediment sampled on the side (I) on the middle (II) and randomly (III-V). Only the results for pond B are presented since no hatching was observed in pond A.

DISCUSSION

Most studies on dormancy (i.e., diapause and quiescence) in copepods have been conducted in temperate regions and at higher latitudes. Only few studies have investigated subtropical waters (Marcus, 1996) and tropical waters (Dahms et al., 2006; Schipp, 2006). Several signals (external or internal signals) can induce the dormancy in copepods such as temperature, photoperiod, food conditions or population density (Dahms, 1995). In the present study, we observed the occurrence of a dormant stage in tropical copepod eggs but further investigations are needed to determine the dormancy type, for instance, to improve the protocol of storage. Subitaneous eggs that usually hatched immediately after spawning can become quiescent under environmental conditions beyond the optimum such as low temperature, food shortage or low oxygen levels (Uye & Fleminger, 1976; Castro-Longoria & Williams, 1999), and be mistaken for diapausing eggs that need to go through a refractory phase before hatching. Subitaneous eggs lack the thick shell of resting eggs and thus they are more sensitive to extreme environmental changes, such as temperature, desiccation, or pH level. During incubation at 30°C we observed the development of mouldy micro-organisms on the top of samples from pond A from which no copepod eggs have hatched. Unlike other studies that extracted the eggs from the sediments (Onbé, 1978; Glippa et al., 2011), here we incubated the whole sediment containing the eggs to keep the same conditions as those in situ. In warm conditions, the micro-organisms present in the sediment can develop rapidly in the sample and this contamination may have affected the quality of the sediment and inhibited the hatching of the eggs. In this study, we assumed that the eggs were quiescent subitaneous eggs for which quiescence has been induced by the low experimental temperature. Nevertheless, among the most important factors inducing dormancy in the field, food conditions and population density are likely to induce dormancy in the ponds since temperature and photoperiod variations are not important in tropical environments. Thus, additional investigations are necessary to confirm the statement that dormancy has been laboratory induced.

In Taiwan, the tropical copepods are exported as deep-frozen food source in aquaculture. However, the use of frozen copepods as food has several limitations compared to live ones. The frozen copepods have less nutritional value compared to live organisms (Ma et al., 2005) and do not stimulate the predatory behaviour of some fish larvae to elicit a feeding response. The living copepods can be introduced and maintained inside the aquariums/ponds or be grown outside in stock cultures in several ways (Ma et al., 2005). Our research leads to an alternative to frozen tropical copepods, using the eggs of a species of the genus *Acartia* that is an important component of the diet of larvae of several commercial fish (Schipp et al., 1999). Here, we found that the eggs of *Acartia bilobata* can be stored up to 3 months at low temperature before hatching is induced. During the hatching period, the abundance is high and can reach up to 12.89 $\times$ 10^9 m^{-3} eggs with a fast potential recruitment of more than 60% after 5 days. These concentrations are very high compared to other coastal and estuarine sites (see table 3 in Glippa et al., 2011). This means that the high density of copepods observed in mass aquaculture ponds could explain the high rate of egg accumulation in the sediment. The presence of a substantial quantity of dormant eggs in tropical copepod mass aquaculture ponds may play an important role in governing the copepod population dynamics of the ponds. Although the aquaculture ponds are relatively easy and costless for producing copepods in tropical regions, the high variability between ponds (i.e., no hatching from pond A), as well as the differences of the reproductive strategies between the key copepods encountered in these ponds should be considered in future studies.

In conclusion, our research leads to an alternative way of copepod exportation for commercial purpose in tropical regions and reorient some researches on the use of copepods in aquaculture. Particularly the freely spawning copepod *Acartia bilobata* is an emerging species of high potential for aquaculture (Pan et al., 2013) and can be studied extensively in the future under controlled conditions (i.e., laboratory), as well as in aquaculture ponds.

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