Hydrobiologia (2010) 652:277–288
DOI 10.1007/s10750-010-0359-8

PRIMARY RESEARCH PAPER

Impacts of male and food density on female performance in the brackish cladoceran *Daphniopsis australis*

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Received: 6 January 2010 / Revised: 18 June 2010 / Accepted: 19 June 2010 / Published online: 11 July 2010

Abstract The roles of male and food density in regulating female performance were investigated in the brackish cladoceran, *Daphniopsis australis*. Parthenogenetic females and ephippial females were tested using a 2 × 4 factorial experiment involving the presence and the absence of a male cross-classified with nil, low, medium and high food densities. For parthenogenetic females, the male presence and food density failed to trigger the switch from asexual to sexual reproduction, but the presence of male negatively affected parthenogenesis through egg abortion. Food density affected the animal longevity but depended on the male presence. The reproductive output was favoured by increasing food densities, but the male presence increased egg abortion, suggesting male being an added stress factor to parthenogenetic females. For ephippial females, food densities affected the frequency of switch from sexual to asexual modes in the absence and the presence of a male. However, the male enhanced switch frequency under low and high food densities. Longevity was increased with the male presence but was unaffected by food density. The ephippial females successfully produced diapausing eggs with the male presence. Although, ephippial females could switch to parthenogenesis but the reproductive output of switched ephippial females was inferior to that of parthenogenetic females since birth. The results reveal that the male presence and food density can impact the performance of female *D. australis*. Hence, this study provides an insight into the understanding of the reproductive biology of cladocerans and a possible alternative explanation for population dynamic of this species and other cladocerans in the field.

Keywords *Daphniopsis australis* · Reproductive mode · Longevity · Reproduction · Parthenogenesis

Handling editor: P. Spaak

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Introduction

Parthenogenesis is a typical reproductive mode in cladoceran life history (De Meester et al., 2004). Under favourable conditions of food, temperature,

and low population density, parthenogenesis is the prevailing mode of reproduction in cladocerans (Shan, 1969; Gilbert & Williamson, 1983) and enables fast population growth (Shrivastava et al., 1999) and rapid resource exploitation (Tomlinson, 1966). However, when the environmental conditions become unfavourable, parthenogenetic females can switch to sexual reproduction through the production of ephippial females (Gilbert & Williamson, 1983). In sexual reproduction, females parthenogenetically produce a brood containing diploid males, and the ephippial females that produce haploid diapausing eggs to be fertilised by a male (Innes & Singleton, 2000). Diapausing eggs are protected by a modified carapace known as ephippium and are resistant to freezing and desiccation conditions (Berg et al., 2001). Under adverse conditions, males are produced earlier than sexual females (Larsson, 1991; Dole-Olivier et al., 2000), and the productions of males and ephippial females are independent events (Hobaek & Larsson, 1990).

In a parthenogenetic population, females are able to switch from an asexual mode to a sexual mode of reproduction and vice versa depending on environmental factors. Among these factors, food availability is essential because it supplies nutrients for egg formation (Vijverberg, 1989). With abundant food supply, parthenogenetic females usually dominate the population and subsequently expand the size of the population through asexual reproduction by exploiting available resources. However, under starvation, females can switch from a parthenogenetic mode to an ephippial mode for sexual reproduction (D'Abramo, 1980). The sexual reproduction is considered successful after the ephippial female mates with a male and starts to produce diapausing eggs.

The switch of reproductive modes in a female cladoceran has been considered an adaptation to survive in harsh conditions (Bunner & Halcrow, 1977). Besides food availability, changes in photoperiod, temperature and population density also trigger the switch from asexual to sexual reproduction (Banta et al., 1939; Berner et al., 1991; Kleiven et al., 1992). Among these proximate factors, the initiation of sexual reproduction seems to be more responsive to the changes in population density (Innes, 1997) and food availability than other factors (Gibson et al., 1998; Martinez-Jeronimo et al., 2007). Nevertheless, despite the fact that the male is the key element in sexual reproduction, little is still known on its role in regulating cladoceran reproduction.

In a pioneer study, Banta et al. (1939) reported that male presence may not lead to a change in reproductive mode in females when food is abundant. The early production of males is believed to represent a wasteful evolutionary strategy, particularly when these males could not find any sexual females to copulate with (Larsson, 1991). Some studies have demonstrated that males usually appear prior to the ephippial females (Stirling & McQueen, 1986; Schwartz & Hebert, 1987) and that the presence of males may stimulate the production of ephippial females (Innes, 1997). Clearly, these observations suggest that the male may play a role to switch the modes of reproduction in females, and this impact might depend on food availability.

The genus *Daphniopsis* is classified under the family of Daphniidae (Cladocera) (Benzie, 2005), and is globally distributed in saline waters in Asia (Sars, 1903), Australia (Sergeev & Williams, 1985), North America (Schwartz & Hebert, 1987) and South America (Hann, 1986). More specifically, *Daphniopsis australis* has been found in brackish waters with salinities of 4–30 PSU (Sergeev & Williams, 1985). Previous studies on *D. australis* have focused on systematics (Colbourne et al., 2006), diversity (Hebert & Wilson, 2000) and osmoregulation (Aladin & Potts, 1995). However, to our knowledge, information on the reproductive biology of brackish cladocerans is still rare. This study aimed (i) to test whether the presence of a male impacts the reproductive mode of parthenogenetic and ephippial females of *D. australis* and (ii) to investigate the interactive effect of the male and food density on the mode of female reproduction.

Materials and methods

Stock culture conditions

Experimental animals, *D. australis*, were collected from a saline water pond (22 PSU) in the Coorong National Park, South Australia. All the collected individuals (ca. 100 parthenogenetic females) were used to start a static culture in 10-l plastic containers. This method has been used in population dynamic studies of other zooplanktonic taxa (Nandini & Rao,

1997; Nandini & Sarma, 2002). The experimental population has been maintained for over 100 generations in our laboratory since 2005. The rearing medium was prepared using filtered seawater diluted with demineralised water to obtain the desired salinity 22–23 PSU. Animals were fed with microalgae *Isochrysis tahitian* at a density of 10^6 cells/ml, which is in the optimal range for most Daphniidae species (Delbare & Dhert, 1996). The food was given every other day. Environmental variables were controlled at a photoperiod of 12 h light and 12 h dark with a light intensity of 1800 Lux at 20–22°C. In order to avoid overcrowding and population crash, the population density was controlled at <1000 ind/l in the stock culture. Prior to the experiment, an allozyme analysis was conducted to test the genetic diversity and clonal structure of 20 randomly chosen animals. The allozyme data for 35 putative loci showed that these animals belonged to a single genetic clone.

The life cycle of *D. australis*

Through the allozyme analysis, the authors confirmed that the *D. australis* population was derived from the progenies of cyclic parthenogenesis consisting of an alternation of an asexual phase and a sexual phase. In the rearing environment, the asexual phase occurred in a condition similar to the stock culture. During the asexual phase, only female individuals were produced through parthenogenesis. From birth to sexual maturation, a female took 6–7 days with four to five juvenile instars to reach maturity. Adult parthenogenetic females could reproduce approximately 10–12 clutches in the life time with embryonic development of 3–4 days. Upon reaching the senescent stage, the reproduction usually ceased, and the female continued to live with an empty brood chamber until death. The life span of a parthenogenetic female ranged 20–30 days but on a rare occasion, some of females lived up to 60 days.

The sexual phase occurred under unfavourable culture conditions and was triggered by low food density and overcrowding when temperature, salinity and photoperiod were set up at 20–22°C, 22–23 PSU and 12 h light and 12 h dark, respectively. During the sexual phase, parthenogenetic females produce a brood carrying either diploid males or females (Innes, 1997), and some of these females are able to transform into sexual females. The sexual female carrying an ephippium was called an ephippial female. Both the male and ephippial female individuals were involved in a mating process, and the fertilised sexual eggs would then develop into diapausing eggs. The ephippial female of *D. australis* could produce a maximum of two diapausing eggs which were encased in an ephippium and detached from the mother during moulting. During our observations, ephippial females usually were formed in the parthenogenetic mode, and an ephippium was produced before the sexual eggs were released. In addition, ephippial females appeared only for a short period because they would die soon after releasing the ephippium. Thus, the lifespan of ephippial females is much shorter than that of the parthenogenetic females.

Experimental design and procedure

Parthenogenetic females

The maternal effect carried over generations by a parthenogenetic female was eliminated through the experimental procedure (Lynch & Ennis, 1983). A single parthenogenetic female was isolated into a single jar containing 50-ml culture medium until the first generation was born. Neonates were then individually transferred into a new jar containing 50-ml of fresh medium for acclimatisation at low, medium and high food densities. As the media were daily renewed, the animals were allowed to continue breeding in the corresponding food densities until the third generation. Only animals from third generation were used for testing the female reproductive performance. Since proliferation under nil food supply was unlikely, experimental animals were taken among cohorts reproduced under the low food density. Meanwhile, for male production, some of the culture containers were treated with a combination of low food density ($<10^5$ *Isochrysis*/ml) and overcrowding (>1000 ind/l) to simulate an adverse environment (Kleiven et al., 1992).

The reproductive responses of parthenogenetic females to the presence or the absence of a male were separately examined under different food densities. Microalgae (*I. tahitian*) were used at four densities to represent high (2×10^6 cells/ml), medium (1×10^6 cells/ml), low (0.5×10^6 cells/ml) and nil food supply. The selection of algal densities was based on the literature data on food requirements

for daphniid and non-daphniid species (Nandini & Sarma, 2003; Nandini et al., 2009). Adult individuals were selected for this study because they could be differentiated between asexual and sexual modes. Parthenogenetic females were 6–7 day old (i.e. the age at first reproduction). Parthenogenetic females were individually inoculated in a jar containing 50-ml medium with nil, low, medium, high food densities. Then, one male was introduced into each container as the male treatment. In the non-male treatment, two parthenogenetic females were inoculated to equalise the animal density. Each treatment was replicated 25 times, and the culture medium was daily renewed. The females were checked daily under a stereomicroscope for sexual switch between asexual and sexual reproductive modes (%), longevity (day), egg production (egg/female) and offspring production (offspring/female). Abortion frequency (%) was also recorded when premature embryos were released from the brood chamber. Any newly hatched neonates were enumerated and immediately removed to offset the crowding effect. Other environmental factors such as temperature, salinity and photoperiod were controlled at the same condition as the animals in the stock culture.

Ephippial females

In the stock culture, three food densities were used to reduce the confounding factors when the animals were transferred to the three respective food densities in the experimental trial. The ephippial females were randomly chosen from the culture containers at each food density. According to the culture history, the ephippial females were over 15 days old. The production of ephippial females in the containers occurred when the density of animals reached >1000 individual/ml in each container. The responses of ephippial females to the presence or the absence of a male under various food densities were tested in the similar protocol as for the parthenogenetic females. Ephippial females were examined for switch frequency from sexual to asexual mode (%), longevity (day), parthenogenetic egg production (egg/female), parthenogenetic offspring production (offspring/female) and diapausing egg production (egg/female). In order to ensure the production of authentic diapausing eggs, each ephippium was checked using a stereomicroscope under 20× magnifications. Any empty ephippium was excluded from the counts. Experiments were continued until all the females had died naturally.

Statistical analysis

The impacts of male and food density on parthenogenetic and ephippial females were tested separately. The abortion and switch frequencies were analysed using *G*-test for goodness-of-fit (Sokal & Rohlf, 1995). Other variables were analysed using two-way ANOVA. In order to interpret the strength between the independent variables, we compared the size effect using Partial Eta Squared test (partial η^2) (Leech et al., 2008). In the parthenogenetic female trial, a square root transformation was used for egg production and total number of offspring to abide by the assumption for variance analysis. A post-hoc comparison was conducted using Tukey HSD test when the significance of the main effects or their interactions were at $P < 0.05$.

In the ephippial female trial, two-way ANOVA was employed, and Tukey HSD test was conducted for post-hoc tests. Inverse transformation was used for data on the number of parthenogenetic eggs to satisfy the normality assumption. In post-hoc comparisons, the non-parametric Games–Howell test was used when data significantly showed heteroscedasticity (Leech et al., 2008). However, in the analysis for the diapausing eggs and parthenogenetic offspring, we dropped the male factor from the two-way ANOVA model because it did not produce any impact. Thus, one-way ANOVA and Games–Howell tests were used to examine the effect of food densities on these two variables. The level of significant differences was also set at $P < 0.05$.

Results

Parthenogenetic females

We followed each animal until its death, but the physiological status varied between treatments. When the male was present, the percentages of dead females carrying eggs at nil, low, medium and high food densities were for 44, 32, 52 and 56%, respectively. Likewise, in the absence of a male, the percentages of

dead females carrying eggs were 20, 32, 72 and 68% with respect to food densities.

Reproductive mode and abortion

None of the parthenogenetic females switched to a sexual mode of reproduction. All parthenogenetic females stayed in an asexual mode until death but the females aborted their eggs at the medium and high food densities (Fig. 1a). At high food density, the abortion frequency was significantly higher in the presence of a male than without a male (*G*-Test; $P < 0.05$; Table 1) while the impact of male presence was insignificant at the medium food density ($P > 0.05$; Table 1). In contrast, in the presence of a male, the abortion frequency significantly increased when the food density changed from medium to high levels ($P < 0.05$; Table 1), but the abortion frequency was unaffected by the food density in the absence of a male ($P > 0.05$; Table 1). Abortion did not occur in parthenogenetic females under nil food or low food density, regardless of the male factor (Fig. 1a).

Longevity

In parthenogenetic females, there was a significant effect of food density, male presence and their interaction (two-way ANOVA; $P = 0.001$; Table 2). The impact of food (partial $\eta^2 = 0.480$) on longevity was stronger than the male (partial $\eta^2 = 0.216$; Table 2). The longest longevity occurred at low food and medium food densities (Tukey HSD; $P < 0.05$; Fig. 1b) regardless of the male factor. The female longevity decreased in the absence of food, but at the low and high food densities, the presence of a male significantly reduced the female longevity ($P < 0.05$; Fig. 1b).

Egg production

In parthenogenetic females, there was a significant interactive effect between the male and food treatments on egg production (two-way ANOVA; $P = 0.001$; Table 2), with food density (partial $\eta^2 = 0.749$) producing a stronger impact than the male (partial $\eta^2 = 0.231$; Table 2). Irrespective of the male, the egg production was higher at the medium food density than other food densities (Tukey HSD; $P < 0.05$; Fig. 1c). Male presence significantly reduced the egg production in the nil food and medium food densities ($P < 0.05$; Fig. 1c), but did not affect the egg production in the low and high food densities ($P > 0.05$; Fig. 1c).

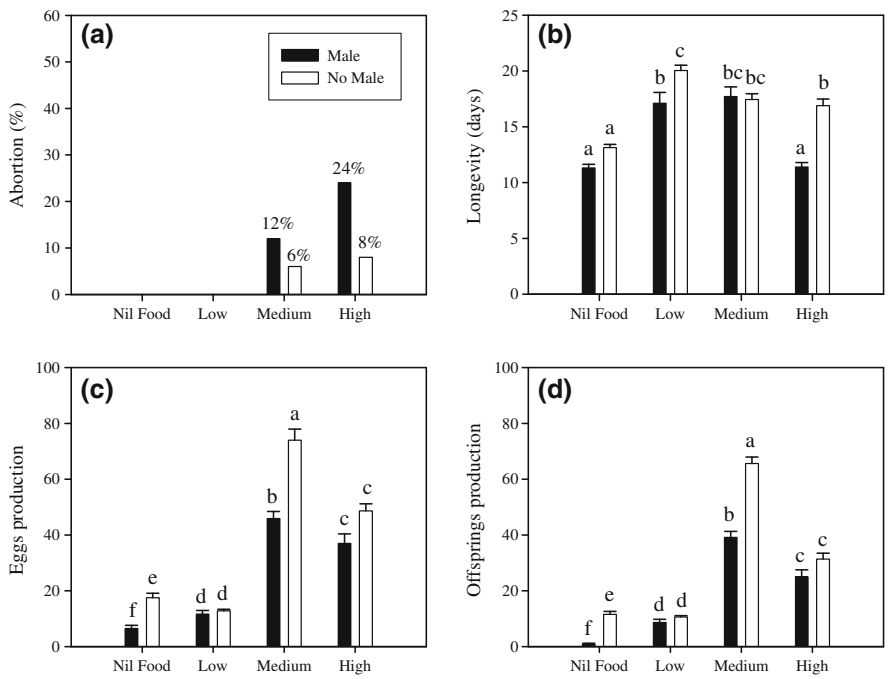

Fig. 1 Abortion frequency (%, **a**), longevity (days, **b**), egg production (eggs/female, **c**) and offspring (offspring/female, **d**) in parthenogenetic females with four food densities (Nil, Low, Medium and High) in the presence and absence of a male

Table 1 Results of *G*-tests on abortion frequency in parthenogenetic females based on (a) male impact under varying food densities; (b) food impact in the presence and the absence of a male

G-Test	Parthenogenetic females		
Abortion frequency	df	Values	*P*
(a) Male impact			
Medium food	1	2.039[a]	0.153
High food	1	8.372[a]	0.004
(b) Food impact			
Male presence	1	4.078[a]	0.043
Male absence	1	0.287[a]	0.592

[a] Values computed using Yates' continuity correction

Offspring production

The pattern of offspring production (Fig. 1d) was similar to that of egg production in parthenogenetic females (Fig. 1c). There was a significant interactive effect between the male and food density on the production of offspring (two-way ANOVA; $P = 0.001$; Table 2), with food (partial $\eta^2 = 0.835$) having a stronger impact than the male (partial $\eta^2 = 0.397$). The highest offspring production occurred at the medium food density, but the male presence significantly reduced offspring production (Tukey HSD; $P < 0.05$; Fig. 1d). Similarly, the presence of a male significantly suppressed the production of offspring in the nil food regime ($P < 0.05$; Fig. 1d), but had no impact at low and high food densities.

Table 2 Results of two-way ANOVA on the impact of food and male on longevity, egg production and offspring production in parthenogenetic females

	df	*F*	*P*	Partial η^2
Longevity				
Food	3	59.191	0.001	0.480
Male	1	52.884	0.001	0.216
Food × male	3	10.614	0.001	0.142
Error	192			
Eggs production				
Food	3	190.654	0.001	0.749
Male	1	57.730	0.001	0.231
Food × male	3	5.530	0.001	0.080
Error	192			
Offspring production				
Food	3	323.007	0.001	0.835
Male	1	126.608	0.001	0.397
Food × male	3	16.192	0.001	0.202
Error	192			

Ephippial females

Although each animal was followed until death, the physiological performance of the females differed between treatments. When the male was present, the percentages of dead females carrying an ephippium at nil, low, medium and high food densities were 96, 28, 80 and 48%, respectively. Likewise, in the absence of a male, the percentages of dead females carrying an ephippium at the above food density levels were 96, 88, 72 and 92%, respectively.

Reproductive mode

Ephippial females switched from the sexual mode to the asexual mode (Fig. 2a). The switch frequency of ephippial females was significantly higher in the male presence than the male absence at low and high food densities (*G*-Test; $P < 0.05$; Table 3). Meanwhile, the presence of a male did not affect switch frequencies at nil food and medium food densities ($P > 0.05$; Table 3). Disregarding the male, food density significantly affected the sex switch frequency ($P < 0.05$; Table 3).

Longevity

The effect of male on the longevity of ephippial females was related to food densities (two-way ANOVA; $P = 0.001$; Table 4) with a stronger contribution of the male (partial $\eta^2 = 0.222$) than the food densities (partial $\eta^2 = 0.037$). In contrast, there was no significant difference in longevity between food densities (two-way ANOVA; $P = 0.063$). In the presence of a male, the longevity of ephippial females in the absence of food was significantly higher than that at low and medium food densities (Tukey HSD; $P < 0.05$; Fig. 2b). In the absence of a male, the longevity of ephippial females was not significantly affected by food density ($P > 0.05$; Fig. 2b).

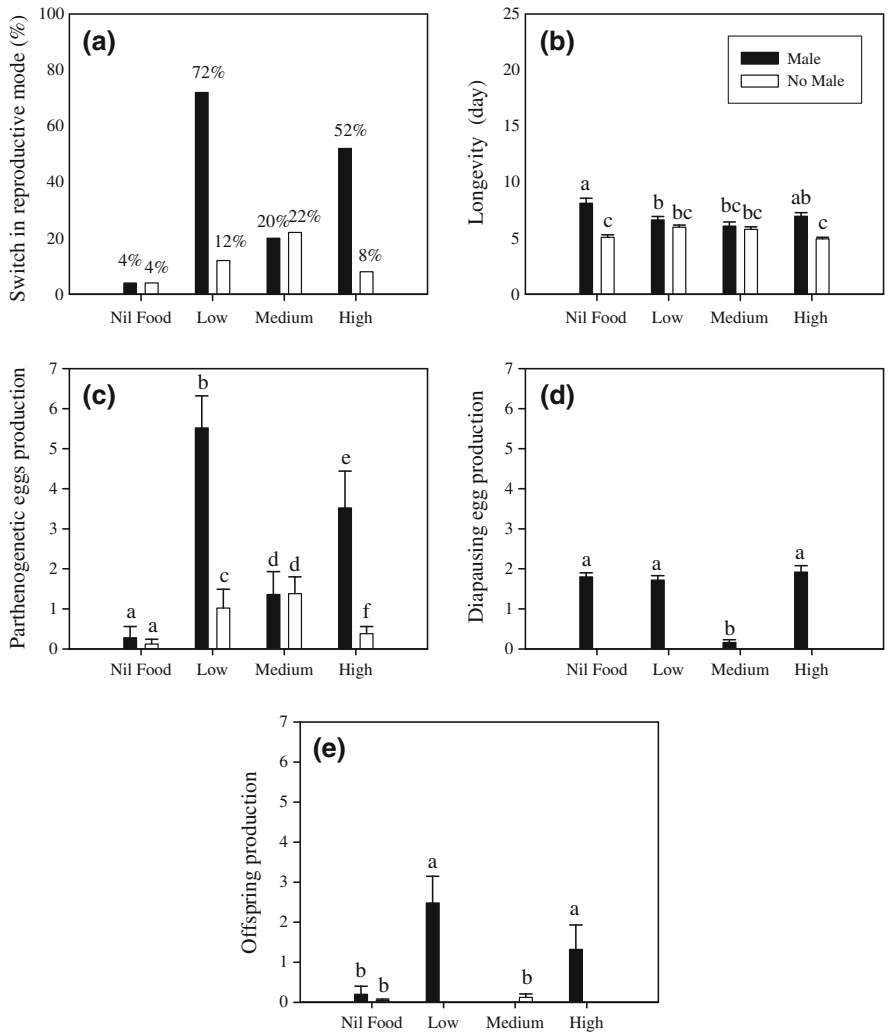

Fig. 2 Reproductive mode switch (%, **a**), longevity (days, **b**), parthenogenetic eggs (eggs/female, **c**), diapausing eggs (eggs/female, **d**) and offspring production (offspring/female, **e**) in ephippial females with four food densities (Nil, Low, Medium and High) at the presence and absence of a male

Table 3 Results of *G*-tests on the switch of reproductive mode in ephippial females based on (a) male impact under varying food densities; (b) food impact at the presence or the absence of a male

G-Tests	Ephippial females		
	df	Values	*P*
(a) Male impact			
Nil food	1	0.001[a]	1.000
Low food	1	47.549[a]	0.001
Medium food	1	0.095[a]	0.758
High food	1	36.057[a]	0.001
(b) Food impact			
Male presence	3	88.857[b]	0.001
Male absence	3	15.309[b]	0.002

[a] Values computed using Yates' continuity correction

[b] Values computed using Williams continuity correction

Table 4 Results of two-way ANOVA on the impact of food and male on the longevity of ephippial females and egg production of the switched ephippial females

	df	*F*	*P*	Partial η^2
Longevity				
Food	3	2.479	0.063	0.037
Male	1	54.774	0.001	0.222
Food × male	3	9.648	0.001	0.131
Error	192			
Parthenogenetic eggs				
Food	3	2.752	0.053	0.149
Male	1	12.722	0.001	0.213
Food × male	3	0.824	0.487	0.050
Error	47			

Egg production

The switched ephippial female referred to an asexual female that was switched from an ephippial female. There was no significant interactive effect between male and food on the production of parthenogenetic eggs in the switched ephippial females (two-way ANOVA; $P = 0.487$; Table 4). However, the male presence significantly enhanced the production of parthenogenetic eggs (ANOVA; $P = 0.001$) under low and high food densities (Games–Howell test; $P < 0.05$; Fig. 2c). The impact of the male on egg production was not detected under nil and medium food densities ($P > 0.05$; Fig. 2c).

Diapausing eggs were only produced by ephippial females in the presence of a male, regardless of food densities (Fig. 2d). However, there was a significant difference in the number of diapausing eggs produced between food densities (one-way ANOVA; $P = 0.001$; Table 5). Diapausing egg production was significantly lower at the medium food density than at other food densities (Games–Howell test; $P < 0.05$; Fig. 2d), but no difference was detected between the nil, low and high food densities (Games–Howell test; $P > 0.05$; Fig. 2d).

Parthenogenetic offspring production

In the switched ephippial females, the production of parthenogenetic offspring was affected by the food density (one-way ANOVA; $P = 0.001$; Table 5). More progenies were produced at low and high food densities than at medium and nil food densities (Games–Howell test; $P < 0.05$; Fig. 2e).

Table 5 Results of one-way ANOVA on the impact of food density on the diapausing eggs of the ephippial females and the offspring production of the switched ephippial females

	df	*F*	*P*
Diapausing egg			
Between groups	3	51.375	0.001
Within groups	96		
Total	99		
Offspring production			
Between groups	4	6.432	0.001
Within groups	120		
Total	124		

Discussion

When *D. australis* entered a sexual phase, the presence of a male is required for mating. In other cladoceran species, the male usually appears prior to the emergence of the ephippial female (Shan, 1969; Stirling & McQueen, 1986; Schwartz & Hebert, 1987). The early appearance of males may stimulate the production of ephippial females and thus males are usually produced before food becomes limited (Innes, 1997). Under this scenario, the presence of males may trigger the switch of females from an asexual to a sexual mode of reproduction, regardless of food conditions. However, in this study, male presence did not change the reproductive mode of asexual female.

Impact of male and food on parthenogenetic females

In our study, no parthenogenetic females switched to ephippial females in any treatments. Instead, all the parthenogenetic females remained in an asexual mode until death. This contradicts the notion that male presence could trigger females to switch from asexual to sexual reproduction (Innes, 1997). Martinez-Jeronimo et al. (2007) reported that the number of males that trigger the sexual reproduction accounted for 6–56% in a cladoceran population, a result consistent with the proportion of males (50%) observed in this study. Despite the lack of switch in reproductive mode, we found that parthenogenetic females showed higher egg abortion at high food density in the presence of a male than that without the male. Abortion in parthenogenetic females can be caused by high temperature (Chen & Folt, 1996), toxic metabolic products (Marques et al., 2004) and low food quality (Urabe & Sterner, 2001). Our study shows that the presence of a male mediated with food supply can also induce female abortion.

The mating behaviour of a male typically starts from pursuing (and eventually copulating with) a female (Damme & Dumont, 2006). We found herein that the male *D. australis* repeatedly approached a parthenogenetic female in an attempt to mate, while the parthenogenetic female consistently refused copulation through escaping. This escape mechanism has been observed in other cladoceran species such as *Chydorus* sp. (Damme & Dumont, 2006), *Daphnia* sp. (Winsor & Innes, 2002) and *Moina* sp. (Forro,

1997), but these studies did not report on the impact of males on the reproductive biology of females. The escape behaviour of parthenogenetic females indicates that the female is not sexually receptive. The constant harassment by a male on an ovigerous female may, however, have led to egg abortion. The abortion rate reached the maximum at the high food density because adequate food facilitated the formation of new clutch of eggs but did not change the magnitude of stress from the mating attempts by a male. However, any stress due to the presence of a male is not strong enough to trigger the production of ephippial females, despite the presence of the male consequently affecting the development of parthenogenesis. This finding implies a unique role of the male in the reproductive biology of female *D. australis*. While the presence of a male is necessary for sexual reproduction, the male presence may also exert adversely impact on the asexual reproduction.

The presence of a male also affected the longevity and reproductive performance of parthenogenetic females, though this impact was dependent on food density. In general, the longevity of a female was significantly enhanced in the presence of food, but the presence of a male reduced female longevity, especially at the low and high food densities. Furthermore, parthenogenetic females produced fewer eggs and offspring in the presence than the absence of a male. It seems that the presence of a male can be an unfavourable factor for asexual reproduction in female and the persistent mating attempts by a male can negatively impact the reproductive output of parthenogenetic females. Therefore, the male presence can induce a considerable amount of stress and consequently reduce the longevity and fitness of parthenogenetic females.

Besides male presence, food availability alone is a cue for switching the reproductive mode in parthenogenetic animals (Carvalho & Hughes, 1983). In response to food availability, the parthenogenetic populations typically switch from an asexual mode to sexual mode in *Daphnia magna* (Carvalho & Hughes, 1983) and *Moina macrocopa* (D'Abramo, 1980). This is an important adaptation to preserve a population over harsh environments (Gyllstrom & Hansson, 2004). However, the population of *D. australis* does not display such a strategy in response to a reduction of food supply in the absence of a male. A similar pattern was also reported in *Daphniopsis studeri* from Antarctica (Gibson et al., 1998) suggesting that food density is not the only cue to induce the switch from asexual to sexual reproduction.

Under the laboratory condition, we demonstrated that male presence reduced an individual's fitness as measured by abortion of parthenogenetic eggs plus reduction in longevity, whereas food availability increased female fitness. At low food supply, females lived longer with compromised sexual reproductive output. The vulnerability of female *D. australis* to food availability and male interference may provide a new insight into the understanding of seasonal succession and population dynamics of this species in nature. However, the relative importance of male and food availability in a natural population may vary, given the complex interactions of other ecological factors in the field. Therefore, the implications of our laboratory results of the population dynamics of *D. australis* to a natural population in the field warrant further study.

Impact of male and food on ephippial female

Parthenogenesis is an adaptive strategy of reproduction under favourable conditions (De Meester et al., 2004). However, it is not clear whether the ephippial female can switch back to parthenogenesis when the condition becomes favourable. When meiosis is involved, returning to a mitotic mode of reproduction may be difficult (Corley & Moore, 1999). In this study, we found that *D. australis* was able to switch from a sexual mode to an asexual mode of reproduction. However, the driving mechanisms are still unclear, and further investigations would be needed to assess this specific issue. Switches also occurred under nil and medium food densities, but this incidence was regulated by the male presence. It seems that the switch of reproductive mode is more sensitive to the male presence in food-constrained females than in non-food-constrained females. Our data suggest that the switch from the sexual mode to asexual mode is more likely under a synergistic effect of unfavourable food supply and male presence.

In this study, the sex switches under unfavourable food conditions were coupled with the male presence.

The switch by an ephippial female to a parthenogenetic mode may provide a greater opportunity to produce more progeny than switching to a male (Lynch, 1989). However, in the absence of a male, this reproductive strategy was not observed under low food density. It seems that the male presence is likely to trigger the switch from the sexual mode to an asexual mode in the ephippial female. The active role of male in sexual reproduction was coupled with the behaviour of avoiding mating in ephippial females, implying that the mating pressure from a male may stress the ephippial female and this stress could facilitate the change of reproductive mode. To our knowledge, this study was the first to report that male interference can stimulate an ephippial female to switch to a parthenogenetic mode.

In such cases, ephippial females generally lived longer in the presence of a male. Usually, the ephippial females of this species produce a maximum of two diapausing eggs in sexual reproduction (Sergeev & Williams, 1985). Ephippial females will produce an empty ephippium when the male is absent (Winsor & Innes, 2002). In our study, diapausing eggs were only found in ephippial females in the presence of a male. The total number of diapausing eggs produced by ephippial *D. australis* in a lifetime was never more than two, suggesting that every mating effort does not always lead to the production of diapausing eggs. The reason why the second cycle of sexual reproduction was missing was probably because the ephippial females either died or switched to an asexual mode of reproduction after the first cycle of diapausing egg production.

Regarding food densities, ephippial female *D. australis* displayed a high frequency of switching at an abundant food supply. This result was not surprising since parthenogenesis usually prevails under abundant food conditions in the life cycle of parthenogenetic animals (Gilbert & Williamson, 1983). The switch from sexual to asexual reproduction is also demonstrated by *Pleuroxus denticulatus* (Shan, 1969) and *D. magna* (Zhang & Baer, 2000). The restoration to parthenogenesis in the ephippial females in both populations was not only due to food availability but also in response to chemical stimuli. In our study, the switch of *D. australis* to parthenogenesis at abundant food could be a strategy to exploit food resource and such a switch consequently increased the reproductive output through parthenogenetic reproduction. However, as ephippial females have a short lifespan, the short longevity limits the duration of asexual reproduction.

In conclusion, the role of males in abortion and in the switching of reproductive mode provides a new and alternative explanation to the population dynamics of cladocerans. The male presence increased egg abortion in parthenogenetic female. The switched ephippial females only produced parthenogenetic females, but did not produce males. Parthenogenetic females failed to produce ephippial females, suggesting that more complex factors regulate the formation of ephippial eggs in female *D. australis*. The intermediate result of ephippial formation did not occur in the medium food density, indicating that food itself is not the only determinant for ephippial formation.

Acknowledgements This research was partially supported by a Scholarship from Universiti Teknologi MARA Malaysia and Malaysian Ministry of Higher Education. Professor Seuront is the recipient of an Australian Professorial Fellowship (project number DP0988554).

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