

Getting ready for mating: The importance of male touching as an accelerator of ovarian growth in a caridean shrimp



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ABSTRACT

The present study was aimed at evaluating the effect of male presence on ovarian maturation in juvenile females and the role of potential chemical, visual and tactile cues emitted by males in that physiological process. A highly gregarious caridean shrimp with sexual dimorphism, *Neocaridina davidi*, was used as experimental model. We tested the hypothesis that male presence accelerates ovarian maturation, mainly through chemical cues. Two experiments were performed. In Experiment 1, juvenile females were reared with adult males, adult females or alone, allowing full contact among shrimps. In Experiment 2, these treatments were evaluated allowing chemical and visual communication, only visual communication, or only chemical communication among shrimps. In both experiments juvenile females were observed once a week under a stereomicroscope to determine ovarian growth rate. Although male presence was not necessary for ovarian maturation, it clearly accelerated the rate of ovarian growth, particularly in the last maturation phase. This led to relatively longer mature ovaries with higher lipid content. On the contrary, the presence of adult females delayed ovarian maturation in juvenile females, while females reared alone showed an intermediate ovarian growth. All these results suggest that adult males release certain cues that stimulate ovarian maturation, while adult females release cues that delay this physiological process. Neither visual cues nor chemical cues released at a distance from females were responsible, either alone or in combination, for the observed effects. Ovarian growth was only influenced when shrimps were allowed to interact freely, probably because of the “mounting” behavior of males towards females. Tactile cues and/or potential chemical cues released by males during this behavior may mediate male stimulatory effect on ovarian growth. Altogether, present results partially support our initial hypothesis and contribute to increase the limited amount of information available on the role of intraspecific multimodal communication in non-behavioral reproductive processes in invertebrate species.

1. Introduction

Numerous aspects of crustacean reproduction are determined by intraspecific communication, which involves chemical, visual, acoustic and tactile cues, emitted either simultaneously or alternately by the organisms (Salmon, 1983; Hughes, 1996; Bushmann, 1999). Visual sexual communication relies on color, shape, size and morphological structures of the body, often in connection with elaborate courtship behaviors (e.g., Hughes, 1996; Latruffe et al., 1999; Christy et al., 2003). These visual cues are usually accompanied in the aquatic environment by chemical ones (Salmon, 1983), as reported in decapods (Hughes, 1996; Acquistapace et al., 2002) and smasher stomatopods (Christy and Salmon, 1991; Marshall et al., 1999). Chemical cues

include sexual pheromones, which may elicit behavioral responses (releaser effects), longer-lasting physiological changes mediated by hormones (primer effects), or both (Salmon, 1983).

Most of the studies addressing chemical communication has focused on releaser pheromones, and widely demonstrated the existence of distance and contact pheromones, released either by females or males, that allow and facilitate the location of sexual partners and mating (e.g. Karplus et al., 2000; Acquistapace et al., 2002; Hardege et al., 2002; Kamio et al., 2002; Mathews, 2003; Díaz and Thiel, 2004; Raethke et al., 2004; Caskey and Bauer, 2005; Berry and Breithaupt, 2010; Zhang et al., 2011; Sal Moyano et al., 2014). Much less emphasis has been placed on pheromones priming physiological processes related to reproduction, and few studies suggested the existence of primer

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pheromones in crustaceans. Atema and Steinbach (2007) proposed that the nuptial molt, which is associated with mating and spawning in some crustacean species, is induced by male chemical cues in the lobster *Homarus americanus*. In addition, ovarian maturation has been proposed to be accelerated by certain pheromones produced in the testes and vas deferens of the caridean shrimps *Paratya compressa* and *Macrobrachium kistnensis* (Takayanagi et al., 1986; Nagabhushanam et al., 1989). However, little is known about the potential role of different cues involved in intraspecific communication as modulators of ovarian development. This physiological process involves the allocation of biochemical reserves to the ovary for yolk synthesis, which supports and influences embryonic development, neonatal growth and survival (George et al., 2001; Izquierdo et al., 2001; Johnston et al., 2007).

Neocaridina davidi is a freshwater caridean shrimp that shows sexual dimorphism, females being larger and more intensely red-colored than males. The ovary is visible through the exoskeleton during the entire maturation process, which allows determining the stage of ovarian development in living females. The species has a “pure searching” mating system (Vazquez et al., 2017; Sganga et al., 2018): shrimps occur in high-density aggregations with males checking other members of the population for sex and receptivity. Newly molted females are quickly copulated and abandoned (Correa and Thiel, 2003). These reproductive events are probably driven by distance and contact pheromones, as reported for other freshwater and marine carideans (Karplus et al., 2000; Mathews, 2003; Díaz and Thiel, 2004; Caskey and Bauer, 2005; Zhang et al., 2011).

Previous observations in the laboratory suggest that males may have some influence on ovarian maturation, since *N. davidi* females reared in the absence of males showed immature ovaries, despite their “adult” weight (from 50 mg according to Tropea et al., 2015). In this context, *N. davidi* becomes an excellent model to study the role of intraspecific communication in physiological processes associated to reproduction in crustaceans. Hence, the objectives of the present study were to determine whether male presence affects ovarian maturation in juvenile females of this species and evaluate the role of potential chemical, visual and tactile cues emitted by males in that physiological process. We tested the hypotheses that male presence accelerates ovarian maturation and that this process is mainly influenced by male chemical cues.

2. Materials and Methods

2.1. Experimental specimens

Female and male shrimps used in the present study were obtained from a reproductive stock provided by Acuamanus and Decorpez Aquaria, Buenos Aires, Argentina. The reproductive stock was maintained in dechlorinated tap water (pH 6.8–7.2, hardness 80 mg l⁻¹, as CaCO₃ equivalents) under continuous aeration and at a constant temperature of 27 ± 1 °C. The photoperiod was 14L:10D. Java moss (*Vesicularia* sp.) was provided as substrate. Shrimps were fed daily ad libitum balanced food for tropical fish (Tetracolor®, Tetra GmbH, Melle, Germany), with the following approximate composition: minimum crude protein 47.5%, minimum crude fat 6.5%, maximum crude fiber 2.0%, maximum moisture 6.0%, minimum phosphorus 1.5%, and minimum ascorbic acid 100 mg kg⁻¹. Water was replaced completely once a week. Thirty ovigerous females were obtained from the reproductive stock and maintained in a separate plastic aquarium (33.5 × 25 × 19 cm) until juvenile hatching. Juveniles from ten ovigerous females were reared under the experimental conditions described above until reaching 15–25 mg, when the first and second pair of pleopods showed morphological differences between males and females and allowed juvenile sexing (Shih and Cai, 2007). Only females were selected from this pool of juveniles. Other pool of juveniles hatched from the remaining 20 ovigerous females were reared in several different plastic aquaria (33.5 × 25 × 19 cm) until reaching 60–90 mg in the case of females and 30–60 mg in the case of males. These adult shrimps were used to “accompany” juvenile

females, as described below. According to this scheme, the accompanying shrimps could be related to each other, and so could be juvenile females, but there was definitely no kinship between accompanying shrimps and juvenile females.

2.2. Experimental design

Two experiments were performed. In Experiment 1, we evaluated the effect of male and female presence on the rate of ovarian growth in female juveniles, allowing full contact among shrimps. In Experiment 2, we assessed the communication modality involved in male presence effect, allowing only visual, only chemical or both communication modalities among shrimps.

2.2.1. Experiment 1

Seventy-two juvenile females weighing 19.82 ± 0.39 mg were placed in pairs in plastic aquaria (18 × 12.5 × 12 cm), which were randomly assigned to one of the following treatment:

- *No companion (NC)*: each pair of juvenile females was maintained alone (88.9 shrimps m⁻²);
- *Female companion (FC)*: each pair of juvenile females was maintained with two adult females weighing 75.84 ± 1.52 mg (177.8 shrimps m⁻²);
- *Male companion (MC)*: each pair of juvenile females was maintained with two adult males weighing 48.76 ± 2.14 mg (177.8 shrimps m⁻²).

Each aquarium was a replicate and 12 replicates were used *per* treatment. Shrimps were maintained under the same conditions of water quality, photoperiod, and feeding, as described above, for the entire experimental period.

All aquaria were cleaned and water was completely replaced once a week. At the same time, juvenile females were weighed (wet weight, precision: 0.1 mg) and observed under a stereomicroscope to determine the relative size and color of the ovaries. These features were used to define the stage of ovarian development, based on the four-stage pattern previously described by Tropea and López Greco (2016): stage I, transparent to opalescent/whitish ovary that extends from the posterior portion of the cephalothorax up to the initial or middle portion of the second abdominal segment; stage II, cream ovary that extends from the posterior portion of the cephalothorax up to the middle portion of the second abdominal segment; stage III, yellow ovary that extends from the posterior portion of the cephalothorax up to the posterior end of the second abdominal segment; stage IV, strong yellow ovary that extends from the middle portion of the cephalothorax up to the initial or middle portion of the third abdominal segment (oviducts may turn white at this ovarian stage) (Fig. 1).

Juvenile females were considered to be sexually mature (first maturity) when they reached ovarian stage IV. At that time, they were weighed (wet weight, precision: 0.1 mg) and sacrificed after being cold-anesthetized at -20 °C for 15 min. The ovaries were dissected rapidly under a stereomicroscope and analyzed to determine their lipid content.

For lipid analysis, three or four replicates were used *per* treatment, each replicate consisting of two or three ovaries. Samples were homogenized in a Potter-type homogenizer (Thomas Scientific, Swedesboro, NJ) and lipids were extracted with a chloroform/methanol mixture, following the method described by Folch et al. (1957). All chemicals were of analytical grade. Total lipid concentration in each sample was determined gravimetrically. Values are expressed as µg mg⁻¹ wet weight.

2.2.2. Experiment 2

In this experiment we applied the same companion treatments that in Experiment 1 (*NC*, *FC* and *MC*) and within each companion treatment we tested three different communication modalities (*Visual*

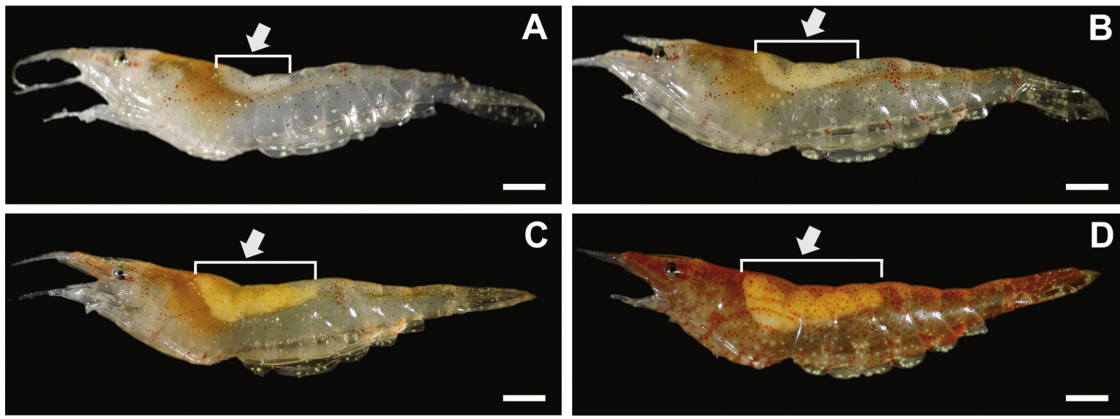


Fig. 1. Stages of ovarian development in females of the freshwater shrimp *Neocaridina davidi*, defined by the relative length and color of the ovary: stage I (A), stage II (B), stage III (C) and stage IV (D). The arrow indicates ovarian location in the female body. Scale bars: 2 mm.

communication, Chemical communication, Visual and chemical communication) with eight replicates per combination of “companion” and “communication modality” (Fig. 2).

Seventy-two plastic aquaria (18 × 12.5 × 12 cm) were divided in two equally-sized compartments, A and B, with a plastic sheet sealed with transparent silicone. Juvenile females weighing 20.16 ± 0.18 mg were placed individually in compartment A of each

aquarium. Compartment B was occupied with no shrimp (NC), with two adult females weighing 81.23 ± 3.57 mg (FC), or with two adult males weighing 40.97 ± 1.43 mg (MC). Within each companion treatment, eight aquaria had compartments A and B separated by a transparent plastic sheet with holes (2 mm in diameter) evenly distributed over the surface, which allowed visual and chemical communication among shrimps. In eight other aquaria the compartments

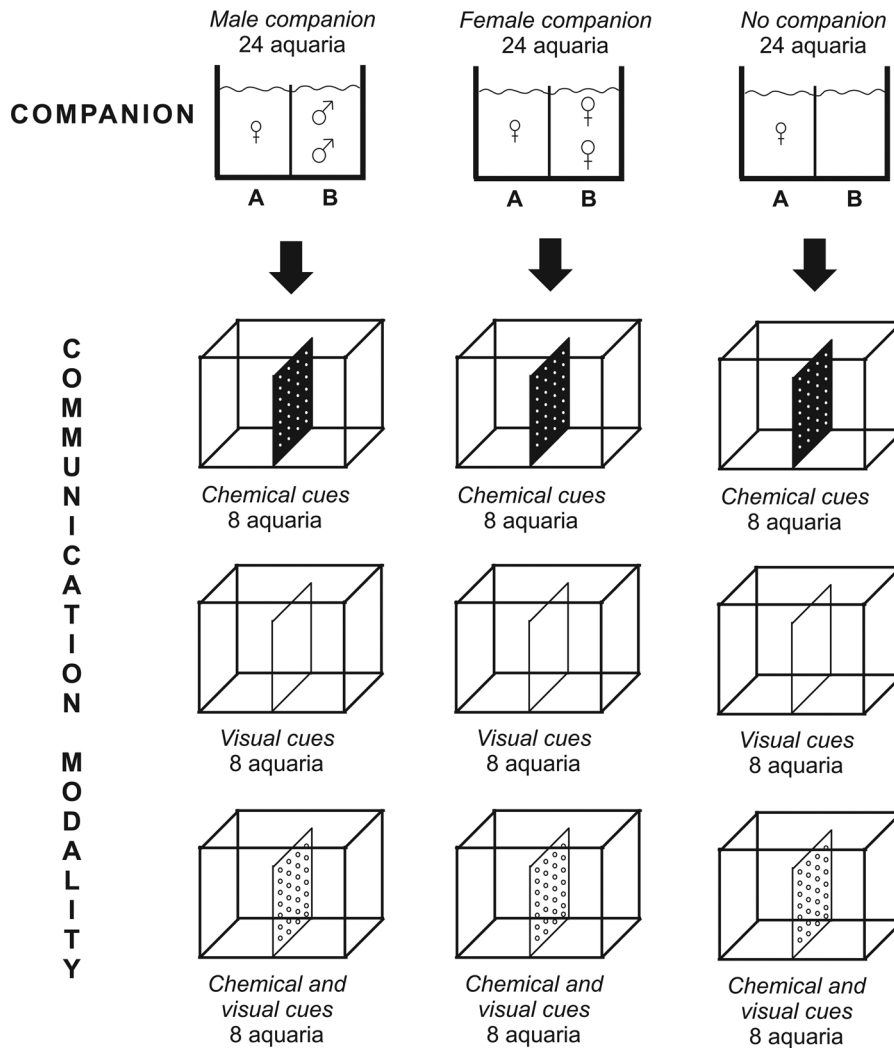


Fig. 2. Scheme of the experimental design applied in Experiment 2.

were separated by a black plastic sheet with holes (2 mm in diameter) evenly distributed over the surface, which allowed only chemical communication among shrimps. In these two sets of eight aquaria each a water flow from compartment B to compartment A ensured the diffusion of potential chemical cues from accompanying males and females to juvenile females. In the remaining eight aquaria, compartments A and B were separated by a transparent plastic sheet with no holes in the surface, which allowed only visual communication among shrimps (Fig. 2).

Shrimps were maintained under the same conditions of water quality, photoperiod, and feeding, as described above, for the whole experimental period, which lasted 60 days. This period was defined based on a previous study showing that *N. davidi* reaches sexual maturity 50 days after hatching at $28 \pm 1^\circ\text{C}$ (Tropea et al., 2015). In addition, most juvenile females reached sexual maturity (i.e. ovarian stage IV) by day 42 of Experiment 1 in the presence of males. All aquaria were cleaned and water was completely replaced once a week. At the same time, juvenile females were weighed (wet weight, precision: 0.1 mg) and observed under a stereomicroscope to determine the relative size and color of the ovaries. As mentioned above, juvenile females were considered to be sexually mature (first maturity) when reaching ovarian stage IV.

2.3. Statistical analyses

The following variables were measured and calculated to analyze ovarian growth in juvenile females from each treatment within Experiment 1 and 2:

- *Percentage of females with mature ovaries (%)*: $100 * (\text{number of females that reached ovarian stage IV} / \text{number of surviving females})$;
- *Female weight at first maturity (mg)*: weight of juvenile females when

they reached ovarian stage IV;

- *Ovarian relative length at each stage (%)*: $100 * (\text{ovarian length} / \text{female length from the tip of the rostrum to the posterior edge of the telson})$;
- *Ovarian growth rate (%/day)*: $(\text{relative length of stage IV ovary} - \text{relative length of stage I ovary}) / \text{number of days elapsed between the onset of experiment and female maturity}$;
- *Ovarian growth rate in the week prior to reaching stage IV (%/day)*: $(\text{relative length of stage IV ovary} - \text{relative length of stage III ovary}) / 7 \text{ days}$;
- *Concentration of total lipids in the ovaries* (Experiment 1): $\mu\text{g lipids} / \text{ovary wet weight}$

All these variables, except the percentage of mature females, were compared among treatments with one-way analysis of variance (ANOVA) for Experiment 1 and two-ways ANOVA for Experiment 2, followed by Tukey test when significant differences were found. Data were checked for normality and equality of variances prior to analyses; these assumptions were met in all cases. Fixed factors were: *Companion* with three levels (no companion, female companion and male companion) in one-way ANOVA; *Companion* and *Communication modality* with three levels (chemical communication, visual communication and both) in two-ways ANOVA. To account for multiple testing, we used the Bonferroni correction and considered significant only those comparisons for which $p < 0.05/7 = 0.007$, seven being the total number of ANOVAs performed in each experiment. This correction is known to be conservative and thus “over-corrected” the raw p -values. The tests for the analysis of ovarian lipid content were carried out at 95% significance level. Finally, the percentage of females that reached sexual maturity within each treatment was analyzed with the Fisher exact test. Results *per* treatment are presented as means \pm SE. All tests were carried out with Infostat® 2016 software (Infostat Group, FCA UNC,

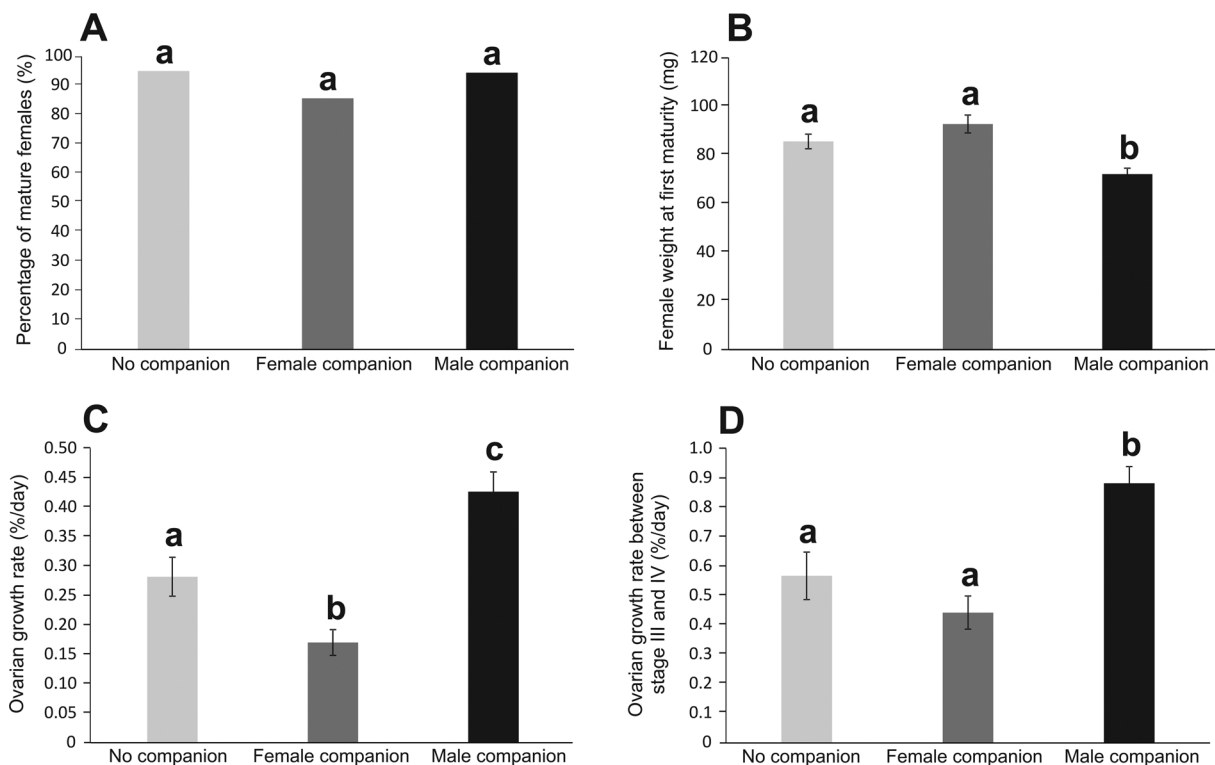


Fig. 3. Sexual maturation in juvenile females of the freshwater shrimp *Neocaridina davidi* reared under different experimental conditions. The percentage of females that reached sexual maturity (A), their body weight at first maturity (B), the ovarian growth rate from stage I (immature) to stage IV (mature) of development (C) and the ovarian growth rate from stage III to stage IV of development (D) were compared among females reared alone (no companion), females reared with adult females (female companion) and females reared with adult males (male companion). Different letters indicate statistically significant differences ($p < 0.007$) among treatments. Results are presented as mean \pm SE, except for the percentage of mature females that lacks SE.

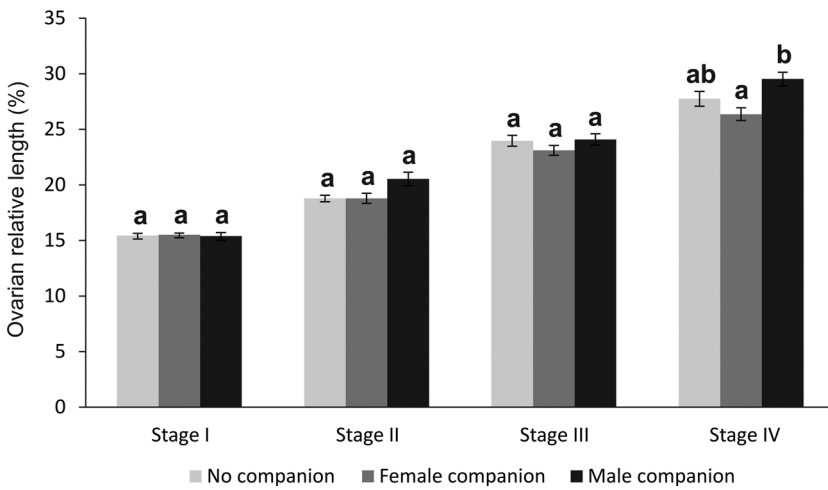


Fig. 4. Relative length of ovaries at different stages in females of the freshwater shrimp *Neocaridina davidi*, maintained under different experimental conditions. Comparisons were made among females reared alone (no companion), females reared with adult females (female companion) and females reared with adult males (male companion) within each ovarian stage. Different letters indicate statistically significant differences ($p < 0.007$) among treatments. Results are presented as mean \pm SE.

4. Discussion

Results from Experiment 1 clearly showed that male presence accelerated ovarian growth rate in *N. davidi* females, particularly during the transition from stage III to stage IV, which is the last phase of ovarian maturation. This led to relatively longer mature ovaries, as compared to females reared with no companion or adult females. As a consequence of this accelerating process, juvenile females reached sexual maturity at a lower weight. These results agree with those previously obtained by Takayanagi et al. (1986) and Nagabhushanam et al. (1989) in the caridean shrimps *P. compressa* and *M. kistnensis*, respectively. However, in those studies male presence seemed to be necessary for ovarian development while in our study male presence had no influence on the percentage of females that reached sexual maturity. The stimulatory effect of males on ovarian maturation is a rather common

phenomenon in vertebrates, and many mammal species show an acceleration of puberty in juvenile females or oestrus induction in adult females by their contact with adult males or their pheromones (Brooks and Cole, 1970; Izard, 1983; Ma et al., 1999; Novotny et al., 1999; Vandenberg, 1999; Murata et al., 2009). The stimulation of sexual maturation has also been demonstrated in fish females exposed to urine or natural pheromone mixtures of males (Chung-Davidson et al., 2013; Huertas et al., 2014). Further studies are necessary to determine the importance of male presence as an accelerator of ovarian growth in crustacean –and invertebrate– groups other than freshwater caridean shrimps.

The presence of *N. davidi* adult females had the opposite effect on ovarian development: although it did not prevent juvenile females from maturing the ovaries, this process was slower and led to relatively shorter mature ovaries than in females reared alone or with males. The

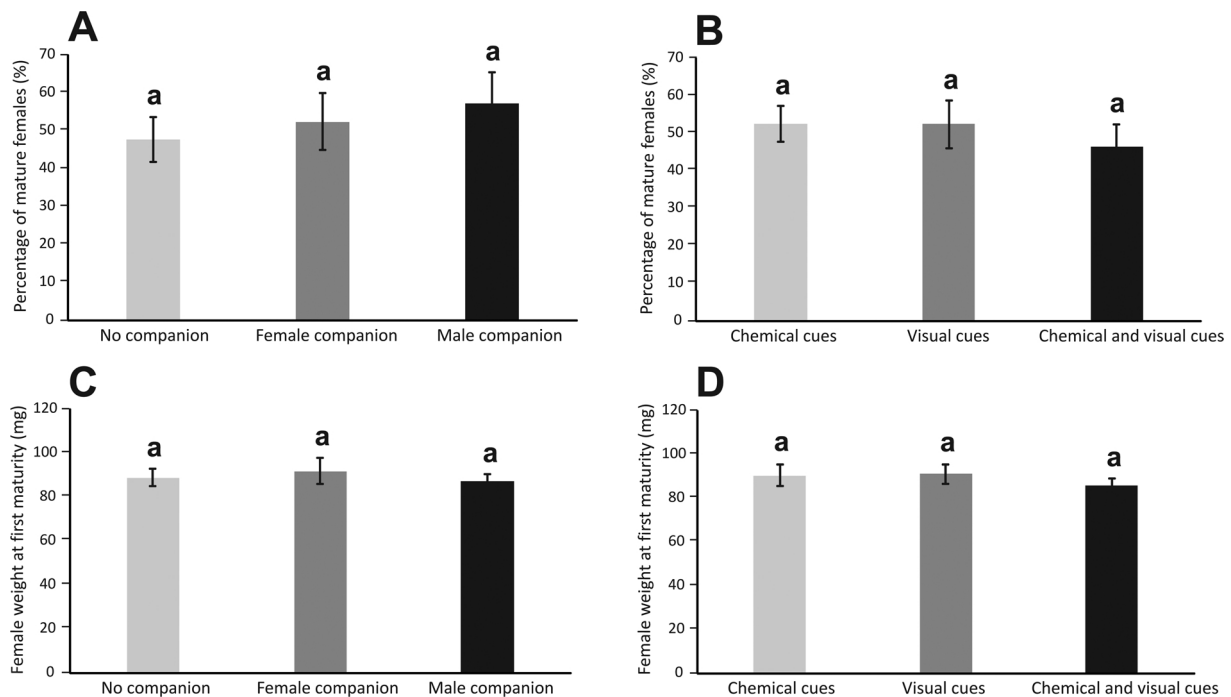


Fig. 5. Sexual maturation in juvenile females of the freshwater shrimp *Neocaridina davidi* reared under different experimental conditions. The percentage of females that reached sexual maturity (A, B) and their body weight at first maturity (C, D) were compared among females reared with no companion (NC), females reared with adult females (FC) and females reared with adult males (MC), with chemical (Ch), visual (Vi) or both cues (Ch + Vi) involved. Different letters indicate statistically significant differences among NC, FC and MC treatments, averaging values for Ch, Vi and Ch + Vi cues (A, C), and among Ch, Vi and Ch + Vi, averaging values for NC, MC and FC (B, D) ($p < 0.007$). Results are presented as mean \pm SE.

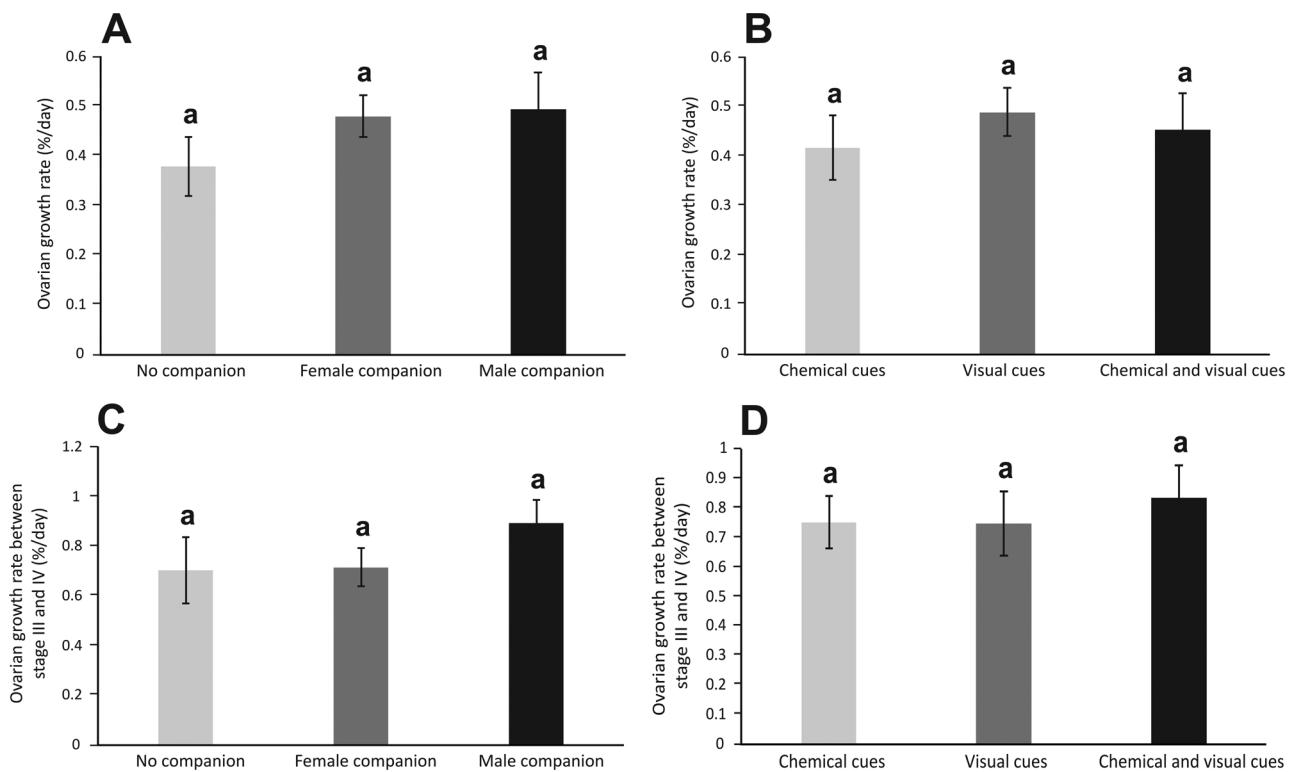


Fig. 6. Ovarian growth in juvenile females of the freshwater shrimp *Neocaridina davidi* reared under different experimental conditions. The ovarian growth rate from stage I to stage IV of development (A, B) and the ovarian growth rate from stage III to stage IV of development (C, D) were compared among females reared with no companion (NC), females reared with adult females (FC) and females reared with adult males (MC), with chemical (Ch), visual (Vi) or both cues (Ch + Vi) involved. Different letters indicate statistically significant differences among NC, FC and MC treatments, averaging values for Ch, Vi and Ch + Vi cues (A, C), and among Ch, Vi and Ch + Vi, averaging values for NC, MC and FC (B, D) ($p < 0.007$). Results are presented as mean \pm SE.

delay in ovarian maturation may have been due to the release of inhibitory cues by adult females, along with the absence of male stimulatory cues, because juvenile females maintained alone showed an intermediate ovarian growth rate. This kind of inhibition is known to occur in some vertebrate species, with young mice females showing a delay in puberty onset when exposed to the urine of grouped adult females (Drickamer, 1977). The effect of inhibitory female cues is more pronounced in other mammal species, such as marmoset monkeys and mole-rats, leading to the complete inhibition of ovulation by the inhibition of gonadotropin secretion (Abbott et al., 1997; Bennett et al., 1997). Similarly, the queen releases pheromones that inhibit ovarian development in workers of some social insects (Endler et al., 2004; Slessor et al., 2005; Wanner et al., 2007; Holman et al., 2010; Matsuura et al., 2010; Trhlin and Rajchard, 2011). However, no previous reports had been made until now on the relationship between inhibitory female cues and sexual maturation in crustaceans. It seems that the cues emitted by adult females of *N. davidi* act only in an inhibitory manner and on juvenile females, since ovarian maturation and spawning are not synchronized in groups of adult females maintained under constant and controlled laboratory conditions (Tropea, pers. obs.). In this context, inhibitory interactions may be a way to delay sexual maturity in juveniles and reduce intrasexual competition. However, information on whether juveniles grow up isolated or in contact with adults and on the operational sex ratio of the species in its natural environment would be necessary to confirm this (Gwynne, 1991; Kraus et al., 1999).

The stimulatory effect of males and the inhibitory effect of females on ovarian maturation may be potentiated in nature by the nearby presence of other males or females, respectively, due to the gregarious behavior of *N. davidi*. This may probably lead to even higher or lower ovarian growth rates than those observed in our study. It is also possible that female and male effects counteract each other if the number of adult males equals that of adult females. However, if the operational sex

ratio is biased towards one sex we would expect a net effect of that sex on the ovarian growth of juvenile females. A greater abundance of mature receptive females may delay ovarian maturation, while a greater abundance of mature males may lead to an acceleration in ovarian maturation. Under this scenario, the predominance of one sex could be an important factor modulating the availability of mature females for reproduction.

Multimodal intraspecific communication has been demonstrated to intervene in diverse behavioral processes associated to reproduction in invertebrates, including courtship displays and mate recognition and attraction (e.g. Christy and Salmon, 1991; Hughes, 1996; Acquistapace et al., 2002; Rybak et al., 2002; Uetz and Roberts, 2002; Takács et al., 2003). However, no reports on the role of multimodal communication in non-behavioral processes have been found to date; hence, the present study provides valuable information on this topic. Interestingly, the variables analyzed in Experiment 2 showed no differences among females reared alone, females reared with adult females and females reared with adult males. These results may indicate that juvenile females were “indifferent” to the presence and sex of their companions, even though they could see them, smell them or both. They also suggest that neither chemical cues released by males at a distance from females (e.g. distance pheromones) nor male visual cues (e.g. transparent coloration) were involved, either alone or combined, in the acceleration of ovarian growth observed in Experiment 1.

One of the closest aquatic species studied to the moment in relation to waterborne chemical signals and reproduction is Mozambique tilapia (*Oreochromis mossambicus*). Unlike *N. davidi* females, pre-ovulated and post-spawning tilapia females show a very rapid increase in the release rate of maturation-inducing steroid hormone when exposed to the urine of dominant males (Huertas et al., 2014). The discrepancy between our study and that by Huertas et al. (2014) may be due to a lack of stimulatory effects of the chemical substances released by *N. davidi* males

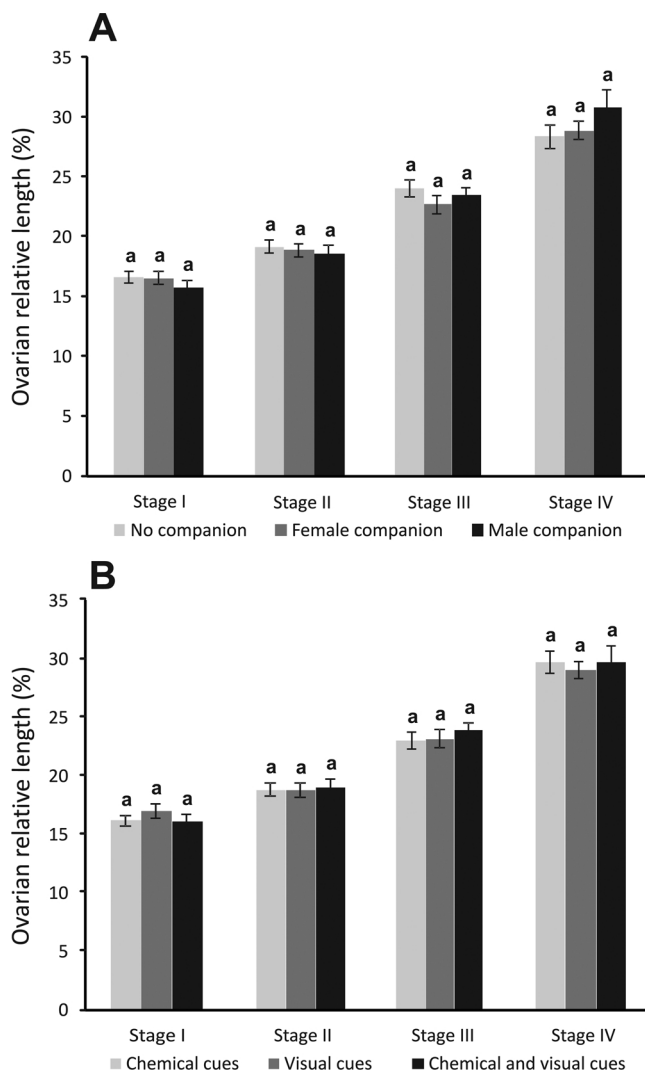


Fig. 7. Relative length of ovaries at different stages in females of the freshwater shrimp *Neocaridina davidi*, maintained under different experimental conditions. Comparisons were made within each ovarian stage among females reared with no companion (NC), females reared with adult females (FC) and females reared with adult males (MC), with chemical (Ch), visual (Vi) or both cues (Ch + Vi) involved. Different letters indicate statistically significant differences among NC, FC and MC treatments, averaging values for Ch, Vi and Ch + Vi cues (A), and among Ch, Vi and Ch + Vi, averaging values for NC, MC and FC (B) ($p < 0.007$). Results are presented as mean \pm SE.

with urine (or across permeable membranes such as gills) on ovarian development. Alternatively, the lack of differential responses to males, females and no companion in compartment B may represent an inability of *N. davidi* females to detect any possible differences in chemicals released to the water by either sex, as previously suggested by Hazlett and Rittschof (1985) for the crayfish *Orconectes virilis*.

Results from Experiment 2 differ from those obtained in Experiment 1, as evidenced by the fact that male presence had a clear effect on ovarian maturation in the latter but not in the former. The discrepancy between experiments suggests that full physical contact between juvenile females and adult males, which was only possible in Experiment 1, was necessary to accelerate ovarian growth. We cannot discard that males contacted females with the tips of their walking legs and/or antennae through the holes of the plastic sheets dividing aquaria in Experiment 2. However, that interaction (if happened) was negligible and had no effect on ovarian growth rate, because no differences were observed between treatments with holes in the dividing sheet (chemical communication alone and chemical communication combined with

visual communication) and the treatment with no holes in the dividing sheet (visual communication alone). When shrimps were allowed to interact freely, a conspicuous and recurrent behavior of males consisting of “mounting” females on their back and touching them with the walking legs over most of their dorsal surface was evident. Females allowed male manipulations with no apparent struggling, similarly to the male-female behavior prior to copulation in the amphipod *Gammarus palustris* (Borowsky and Borowsky, 1987). The behavior showed by *N. davidi* males has been previously reported as a general behavior of caridean shrimp males when encountering a receptive female (i.e. newly-molted female with mature ovary). In that case, male “mounting” behavior is always followed by copulation (Bauer, 2004). However, the novelty our findings is that this same behavior occurred repeatedly *before* female mating molt. This means that males mounted females *before* they mature the ovary and consequently it was not a behavior associated with copulation. Instead, it could be used by males to sense female receptivity, this process being probably mediated by contact pheromones (Bauer, 1979; Seibt and Wickler, 1979; Caskey and Bauer, 2005; Zhang and Lin, 2006).

It is possible that males released certain chemical cues upon physical contact with females and that both factors (tactile and chemical cues) were involved in modulating ovarian growth in freely interacting shrimps. These cues may have been sensed by females through distributed chemoreceptors packaged with mechanosensors into sensilla dispersed over the body surface (Schmidt and Mellon, 2010). In this context, the “mounting” behavior of males may have primarily developed to determine the gender and receptivity of females and to give information on the sender itself (e.g. gender, reproductive state), secondarily serving as a positive stimulus for ovarian growth. Previous studies on mammals have demonstrated that male pheromone stimulation provokes the immediate increase in luteinizing hormone pulse frequency in females, which enhances follicular growth and estradiol secretion (Bronson and Desjardins, 1974; Martin et al., 1986). It is not possible at this stage of our knowledge to determine whether male stimulation triggers in *N. davidi* females the release of gonad stimulating hormone from the brain and thoracic ganglion and/or inhibit the release of gonad inhibiting hormone from the X-organ-sinus gland complex in the eyestalk (Fingerman, 1987). The hormonal mechanisms underlying ovarian growth acceleration under male presence may be the subject of future studies.

Communication relying on physical contact may be facilitated by the gregarious behavior of *N. davidi*, because the probability of encountering (and touching) a conspecific is much higher when living in groups (Chak et al., 2015). Such contact may involve tactile and/or chemical cues. The importance of tactile cues in crustaceans has been also demonstrated for other non-behavioral processes, such as somatic growth. Karplus and Barki (2004) showed that the growth of small individuals of the red-claw crayfish *Cherax quadricarinatus* was reduced by 50% only when in full contact with large individuals. As no growth suppression was observed when only chemical or visual cues were involved, the authors attributed these results to direct competition for food. Considering that food availability affects ovarian maturation (Reimers et al., 1993; Chatzifotis et al., 2011; Charron et al., 2015), and that shrimp density was different among treatments, it may be argued that competition for food also influenced our results. However, this is unlikely because shrimps were fed ad libitum, which guaranteed an equal food availability among treatments. Moreover, the fact that the treatment with the lowest density (i.e. juvenile females reared alone) did not show the highest ovarian growth rate further discards competition for food as a factor influencing ovarian maturation in our study.

Even though adult females of *N. davidi* are clearly distinguishable from adult males based on their coloration and size, these visual features had no influence on the ovarian growth of juvenile females. Communication between sexes was shown to rely on visual cues in species living in tropical waters with usually high visibility, such as the snapping shrimp *Alpheus heterochaelis* (Hughes, 1996). Unlike this

species, *N. davidi* inhabit various inland water bodies with different degrees of turbidity and complexity, such as lakes, ponds, rivers and streams (Weber and Traunspurger, 2016). The fact that visual cues are limited in turbid environments may explain the unaltered maturation rate of juvenile females when only visual communication with adult shrimps were allowed. In case visual cues play a role on ovarian maturation, it may be in combination with tactile and chemical cues (Chak et al., 2015).

Interestingly, total lipid content was much higher in the ovaries of females reared with males than in those reared with adult females or alone. This result suggests that juvenile females were able to modulate the amount of energy reserves allocated to the ovary as a function of their social environment. Females have been shown to allocate their primary reproductive effort (egg number, size or quality) differentially in several taxa according to the attractiveness of their mates (Gil et al., 1999; Reyer et al., 1999; Cunningham and Russell, 2000; Sheldon, 2000; Kolm, 2001; Saino et al., 2002; Kotiaho et al., 2003; Aquiloni and Gherardi, 2008). However, to our knowledge, no previous study has ever reported differential reserve allocation in relation to the presence/absence of males.

The accumulation of nutrients in the developing ovaries has a direct effect on embryonic development, neonatal growth and survival (e.g., George et al., 2001; Izquierdo et al., 2001; Johnston et al., 2007). Lipids, in particular, have an energetic and structural function during lecithotrophic development, and influence some reproductive aspects in crustaceans and fishes, such as egg number and hatching rate (Palacios et al., 2001; Wouters et al., 2001; Bjerkeng, 2008). They are also associated with the physiological condition of larvae and their nutritional vulnerability (Andrés et al., 2010; Urzúa et al., 2012; Rotllant et al., 2014). In this context, the way a female synthesizes egg yolk may have important fitness consequences for her offspring, and mature ovaries with more lipids may be considered of higher quality. However, the allocation of lipids to the maturing oocytes is a costly process for females, which may transfer as much as 60% of their lipid reserves in the hepatopancreas to the ovary (Herring, 1973). The costs associated with ovarian maturation may explain the development of higher quality ovaries when females face favourable conditions for reproduction (i.e. male presence). As in the study by Aquiloni and Gherardi (2008), an interesting question that arises from present results is why females still allocate biochemical reserves and mature their ovaries when they have no chances of raising any offspring due to male absence. It seems that ovarian maturation in *N. davidi* females is triggered independently of social environment, maybe because the probability of encountering no males in nature is very low for this highly-gregarious species.

In conclusion, although male presence was not necessary for ovarian maturation in the freshwater shrimp *N. davidi*, it clearly accelerated the rate of ovarian growth. As a result, females showed relatively longer mature ovaries with higher lipid content. Full physical contact between adult males and juvenile females, and not visual or chemical cues released at a distance from females, may be involved in male stimulatory effect. On the contrary, female presence delayed ovarian maturation in juveniles, probably as a result of inhibitory cues emitted by adult females. Present results support our initial hypothesis stating that male presence accelerates ovarian maturation, but they do not allow us to confirm or reject the hypothesis stating that this physiological process is mainly influenced by male chemical cues. They are, still, of great theoretical interest, contributing to increase the limited amount of information available on male stimulatory effects in invertebrate species.

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