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Cue hierarchy in the foraging behaviour of the brackish cladoceran *Daphniopsis australis*

MCCLOUD Clayton Leigh[1], ISMAIL Hasnun Nita[2], SEURONT Laurent[3, *]

[1] *Australian Fisheries Management Authority*, *Canberra*, *Australia*

[2] *School of Biology*, *Universiti Teknologi MARA*, *Selangor*, *Malaysia*

[3] *CNRS*, *Univ. Lille*, *Univ. Littoral Côte d'Opale*, *UMR 8187*, *LOG*, *Laboratoire d'Océanologie et de Géosciences*, *F 62930 Wimereux*, *France*

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Abstract Zooplankton communities are an essential component of marine and freshwater food webs. However, there is still a relative lack of information on how these organisms behaviourally respond to a range of abiotic and biotic stressors. Specifically, the behaviour of the cladoceran *Daphniopsis australis*, a species endemic to South-eastern Australian saline lakes and ponds, is still unknown despite its potential role in the structure and function of inland water ecosystems. The swimming behaviour of males, parthenogenetic females and epiphial females was investigated under various conditions and combinations of food and conspecific cues. In the absence of cues, males displayed the most extensive swimming behaviour, exploring all areas of the container, and swimming in a series of relatively straight trajectories. In contrast, females typically exhibited a hop-and-sink motion characterised by the alternation between short bursts of swimming and sinking phases. Both females spent long periods near the bottom of the container, but epiphial females appeared to be more active than parthenogenetic ones that rarely made an excursion in the water column. In the presence of cues, males and females showed abilities to detect infochemicals from food and conspecifics, but exhibited specific behavioural strategies. Males essentially increased their swimming speed in the presence of food and/or conspecific infochemicals, and this increase was independent on the source of the cues, i.e. food, conspecific or a mixture of food and conspecifics. In contrast, females exhibited cue hierarchies that were related to their sexual status. Parthenogenetic females swam faster in the presence of food and a mixture of food and conspecific infochemicals than in the presence of cue from the opposite sex, which did not significantly differ from control observations conducted in the absence of cues. Epiphial females decreased their swimming speed in the presence of cues, with the most significant behavioural answers being driven by sex-related cues.

Keyword: zooplankton; chemical cues; sex-specific behaviour

1 INTRODUCTION

Cladocerans are an integral component of the planktonic and benthic crustacean fauna in freshwater ecosystems such as lakes and ponds (Mergeay et al., 2006). These organisms are the primary herbivores of these ecosystems, eating algae and bacteria and contributing to the recycling of nutrients in the water column (Dodson and Frey, 2001). They also provide a large proportion of the diets of planktivorous fish and invertebrates (Brancelj et al., 2012; Bledzki and Rybak, 2016). Cladocerans are subject to both bottom-up and top-down forces, hence play an essential role in carbon and energy transfer through the food web (Brancelj et al., 2012; Bledzki and Rybak, 2016). The interactions of cladocerans with their biotic and abiotic environment at micro-scales (i.e. typically <1 m) have often been overlooked, but can influence the structure of entire ecosystems. As stressed in the context of the behavioural ecology of freshwater and marine copepods, swimming behaviour has increasingly been considered as a stepping-stone to explain phenomena ranging from individual encounter

* Corresponding author: laurent.seuront@cnrs.fr

rates to population dynamics (Kiørboe, 2008; Seuront, 2015a).

The swimming behaviour of cladocerans, in particular *Daphnia* sp. (i.e. the so-called water flea), has received a great deal of attention (e.g. Brewer, 1998; Seuront et al., 2004a, b; Garcia et al., 2007; Da S. Ferrão Filho et al., 2008; Nihongi et al., 2011, 2016; Ziarek et al., 2011; La et al., 2014; Uttieri et al., 2014; Hinow et al., 2015). The regular beating of the second set of antennae enables these crustaceans to swim with characteristic hops (Dees et al., 2008). *Daphnia* swimming behaviour is dependent on body size (Dodson and Ramcharan, 1991) and may also be affected by various factors such as light, water temperature, salinity, and the presence of both food and predators (Baylor and Smith, 1953; O'Keefe et al., 1998; Hamza and Ruggiu, 2000; Ziarek et al., 2011). Noticeably, the alteration of *Daphnia* swimming behaviour under various conditions of chronic water contamination is now acknowledged as one of the most sensitive biomarkers in toxicity assessment; see Bownik (2017) for a review.

In contrast, the behaviour of the genus *Daphniopsis* (Cladocera: Daphniidae; Benzie, 2005) is still unknown despite its global distribution in the inland saline waters of Asia (Sars, 1903), Australia (Sergeev and Williams, 1985), North America (Schwartz and Hebert, 1987) and South America (Hann, 1986). More specifically, *D. australis* (Sergeev and Williams, 1985) is endemic to South-eastern Australia (Hebert and Wilson, 2000), where it is commonly found in ephemeral saline lakes and swamps for salinity ranging from 4 to 30 (Sergeev and Williams, 1985). The occurrence of *D. australis* is highly seasonal, with high and low abundances respectively observed in spring and winter, while it is undetectable during summer and autumn (Campbell, 1994). Previous studies on *D. australis* have essentially focused on their systematics (Colbourne et al., 2006), morphology (Aladin, 1991), diversity (Hebert and Wilson, 2000), seasonal dynamics (Campbell, 1994), biogeography (Timms, 2007), osmoregulation (Aladin and Potts, 1995), thermal and halo tolerance (Aladin, 1991; Ismail et al., 2010a), reproductive biology (Ismail et al., 2010b), and life history as a function of biotic parameters (food quality; Ismail et al., 2011a) and abiotic parameters such as temperature and salinity (Ismail et al., 2011b, c).

As all cladocerans, *D. australis* females reproduce sexually (epiphial female), but when conditions are favourable, asexual reproduction occurs via parthenogenesis. Males and epiphial females are produced by parthenogenetic females when the environment deteriorates due to e.g. overcrowding, starvation or anoxy (Dodson and Frey, 2001). Epiphial females produce resting eggs that are capable of surviving harsh conditions such as summer droughts and winter frost (Schwartz and Hebert, 1987). *D. australis* is one species that has been successful in adapting to the extreme temperature and salinity of the Australian climate. However, despite their potential role in the structure and function of South Australian inland water ecosystems, there have been few ecological studies on this genus and none on this particular species. In this context, the objectives of this study were to quantify the swimming behaviour of *D. australis* males and females (both parthenogenetic and epiphial) in the absence and presence of cues from food and conspecifics to assess if this species has comparable sensory abilities to detect food items, and conspecifics using chemical and/or mechanical cues than the well studied *Daphnia* sp. and freshwater and marine copepods, and the subsequent ability to modify their swimming behaviour accordingly (e.g. Brewer, 1998; Nihongi et al., 2011; La et al., 2014; Uttieri et al., 2014; Nihongi et al., 2016).

2 MATERIAL AND METHOD

2.1 *Daphniopsis australis* culture

Individuals of *D. australis* were cultured in 20-L containers, and fed daily *Isochrysis affinis galbana* (Tahiti isolate, obtained from the Australian National Algae Culture Collection, CSIRO, Hobart, Tasmania) at a density of 10^6 cells/mL, which is in the optimal range reported for most Daphniidae species (Delbare and Dhert, 1996). The continuous culture was maintained under constant conditions of temperature (22°C) and salinity (22) and kept on a 12-h light/12-h dark cycle with an irradiance of 300 W/m^2 (Light Intensity Recorder MDS-MkV, Alec Electronics Inc., Kobe, Japan). In order to avoid overcrowding and population crash, the population density was controlled at less than 1 000 ind./L in the stock culture (Ismail et al., 2010a, b).

2.2 Experimental design and behavioural observations

One control and six different water treatments were used to provide *D. australis* with a hierarchy of cues (Table 1). Control artificial seawater was made with milli-Q water and Instant Ocean Sea Salt (Instant

Table 1 Water treatments used to hierarchically assess the effect of biological cues on the motion behaviour of the cladoceran *D. australis*

Water treatment	Code	Cues
Artificial seawater, control	ASW	None
Filtered *Isochrysis* culture	FIC	Food chemicals
Unfiltered *Isochrysis* culture	UIC	Food chemicals and presence
Filtered artificial seawater conditioned with *D. australis* of the opposite sex at 20 ind./L	ASW_{Da}	Conspecific chemicals
Unfiltered artificial seawater conditioned with *D. australis* of the opposite sex at 20 ind./L	$UASW_{Da}$	Conspecific chemicals and presence
Filtered *Daphniopsis* culture water	FDCW	Food and conspecific chemicals
Unfiltered *Daphniopsis* culture water	UDCW	Food and conspecific chemicals, conspecific presence

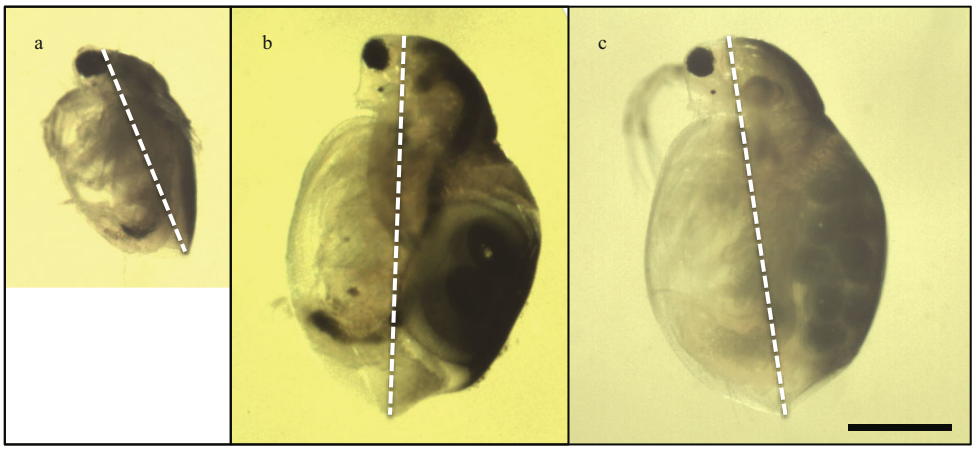

Fig.1 *D. australis* male (a), epiphial female (b) and parthenogenetic female (c)

The dashed white bars indicate how the size of individuals was measured, and the black scale bar indicates 0.5 mm.

Ocean®, Blacksburg, VA, USA) to 22. Treatment solutions based on *I. affinis galbana* culture (hereafter *Isochrysis* culture) and *D. australis* culture (hereafter *Daphniopsis* culture) were made by diluting *Isochrysis* and *Daphniopsis* cultures with artificial seawater at a ratio of 1:1. *Isochrysis* culture water contained both *I. affinis galbana* cells and their exudates. Similarly, *Daphniopsis* culture water contained both *D. australis* and their specific chemicals, and *I. affinis galbana* cells and their exudates. We also created pheromone-conditioned water incubating males, parthenogenetic females and epiphial females in control seawater during 24 h at a concentration of 20 individuals per litre (Seuront and Stanley, 2014). Cues were subsequently hierarchized through behavioural experiments conducted using filtered and unfiltered *Isochrysis* and *Daphniopsis* culture waters, and both pheromone-conditioned seawater in the presence and absence of conditioning individuals (Table 1). All filtrations were conducted through Isopore membrane filters (Millipore; 0.8 μm porosity) using a vacuum air pump. All water types used in the experiments were maintained at the temperature and salinity of the cultures (i.e. 22°C and 22). All water treatments made from *Isochrysis* and *Daphniopsis* cultures were prepared on the same day to avoid any bias related to putative cumulative effects of both *I. affinis galbana* and *D. australis* exudation and excretion.

Individual *D. australis* were collected from the culture using a wide-tip pipette to prevent physical damage to the organisms and identified under a dissecting microscope to determine sex and female stage (Fig.1). Males were smaller (1.00–1.30 mm), whilst both parthenogenetic and epiphial females sizes were similar and ranged between 1.60–2.16 mm and 1.68–2.25 mm, respectively. The three adult types were then separated into 250 mL beakers filled with control seawater, and placed in a culture cabinet (22°C) until used in the video experiments. All video recordings of freely swimming *D. australis* occurred within one hour after the organisms were taken from the culture. For each adult type (n=3) and water treatment (n=7, including control), 3 individuals were transferred into a 3 375-mL (i.e. 15 cm×15 cm×15 cm)

glass behavioural container, placed inside the culture cabinet (22°C) and allowed to acclimatise for 15 min (Seuront, 2006, 2013). This was replicated 13 times for each sex, under control conditions in the absence of any cues, and under each of the six treatment types (i.e. 21 treatments replicated 13 times). Sex and treatments were randomized (Seuront, 2006). Three-dimensional trajectories of *D. australis* were recorded at a rate of 25 frame/s using two synchronized infrared digital cameras (Sony Handycam; DCR-PC120E) placed orthogonally and each facing one side of the experimental container. Six arrays of 72 infrared light-emitting diodes provided the only light source from the bottom of the experimental container (Seuront, 2013). Note that because infrared light-emitting diodes generate heat from the bottom of the experimental container, we gently ventilated the culture cabinet during the behavioural experiments to prevent the formation of a temperature gradient. This was further controlled through measurements of the temperature of the cabinet, and the temperature of the bottom and surface waters of the experimental container at the beginning and the end of the experiments. No significant changes in temperature were ever recorded.

2.3 Image analysis and behavioural analysis

Each behavioural experiment lasted 30 min, after which the resulting 273 video recordings were transferred to a computer, and (x, y, z) coordinates of *D. australis* were subsequently automatically extracted using LabTrack software (DiMedia, Kvistgård, Denmark). All behavioural analyses were based on trajectories in which *D. australis* individuals were swimming at least two body lengths away from a conspecific, and any chamber walls or the surface of the water (Seuront, 2006, 2013). The experiments were consistently run under the same conditions of temperature (22°C) and salinity (22) in the dark and at night (between 22:00 and 02:00) to avoid any potential behavioural artifact related to the diel cycle of *D. australis* (Seuront, 2011). To work on statistically consistent swimming paths, paths of similar durations (i.e. 55–60 s) were selected according to the above-mentioned criteria, and the same number (n=25) of swimming paths was considered for the behavioural analysis of each experimental conditions, i.e. a total of 525 paths.

Daphniopsis australis motion behaviour was subsequently quantified using three metrics widely used in zooplankton behavioural studies: swimming speed, net-to-gross displacement ratio (NGDR) and swimming activity index; see Seuront et al. (2004c) for a review. The maximum speed was also determined. The distance d (mm) between two points in a three-dimensional space was computed from the (x, y, z) coordinates as $d=((x_{t+1}-x_t)^2+(y_{t+1}-y_t)^2(z_{t+1}-z_t)^2)^{1/2}$, where ($x_t$, y_t, z_t) and (x_{t+1}, y_{t+1}, z_{t+1}) are the positions of a *D. australis* individual at time t and t+1, respectively. The swimming speed v (mm/s) was subsequently calculated as $v=df$, where f is the sampling rate of the camera, i.e. f=25 frame/s. The net-to-gross displacement ratio (NGDR) was calculated as NGDR=ND/GD, where ND (mm) is the net displacement of a *D. australis* or the shortest distance between starting and ending points and GD (mm) is the gross displacement of a *D. australis* or the actual distance travelled between starting and ending points. The NGDR describes the linearity of *D. australis* swimming paths, with a high ratio relating to a straight path and a low NGDR relating to a curvy trajectory (Buskey, 1984). The activity of *D. australis* was estimated using an activity index A_i defined as $A_i=100\times t_{track}/t_{swim}$, where t_{track} and t_{swim} are the duration of a swimming path and the time *D. australis* spent swimming, respectively.

2.4 Statistical analysis

Non-parametric statistics were used throughout this work as criteria for normality and homogeneity of variance were not met. Comparisons between sexes and treatments were conducted using the Kruskal-Wallis test (KW test hereafter), and when appropriate were followed by a multiple comparison procedure based on the Tuckey test (Zar, 2010) to identify significant differences between sexes and cue treatments. All statistical tests were run using home-made Fortran routines coded from the procedures described in Zar (2010).

3 RESULT

3.1 Swimming behaviour of *Daphniopsis australis* in the absence of cues

Exemplars of *D. australis* two-dimensional swimming trajectories are shown in Fig.2. Each sex displayed irregular and erratic motions including strong jumps. Males displayed the most extensive style of swimming, exploring all areas of the container, and swimming in a series of relatively straight trajectories (Fig.2a). In contrast, females typically exhibited a hop-and-sink motion characterised by the alternation between short bursts of swimming and sinking phases. Both types of females spent long

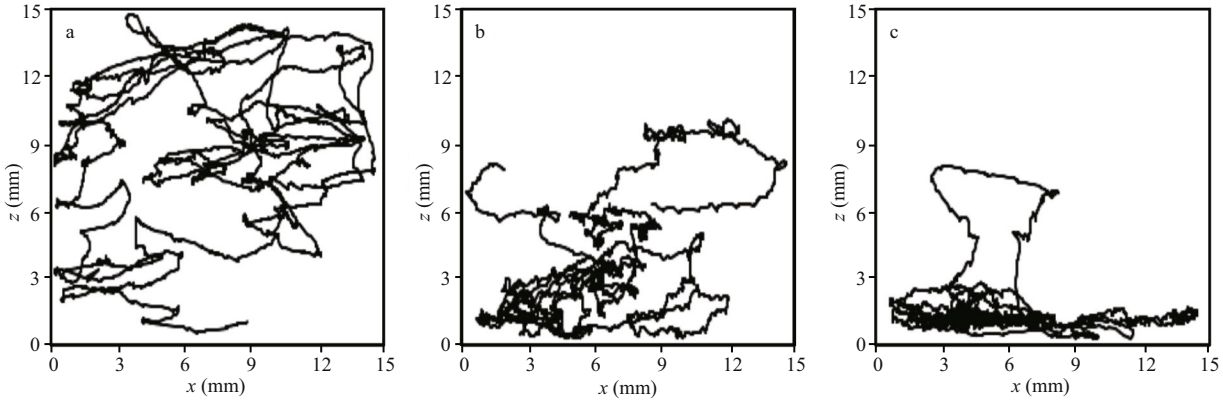

Fig.2 Two-dimensional projections of the typical swimming behaviour exhibited by a male (a), epiphial female (b) and parthenogenetic female (c) of the cladoceran *D. australis* in a 150 mm×150 mm×150 mm experimental container

The three trajectories shown here all have a duration of 60 s.

periods near the bottom of the container, but the epiphial females (Fig.2b) appeared to be more active than parthenogenetic ones who rarely made an excursion in the water column (Fig.2c).

More specifically, *D. australis* swimming speed significantly differs between sexes (KW test, $P<0.05$). A subsequent Tuckey test showed that epiphial females swam significantly faster (7.35±0.26 mm/s; $\bar{x}$±SD) than both males (6.38±0.26 mm/s) and parthenogenetic females (5.73±0.44 mm/s). Note, however, that the maximum swimming speed reached by males was significantly higher ($P<0.05$; 92± 5.8 mm/s) than those observed for both parthenogenetic females (57±5.5 mm/s) and epiphial females (63±3.9 mm/s), which could not be statistically distinguished ($P>0.05$). Finally, both the net-to-gross displacement ratio and the activity index A_i significantly differed between all sexes ($P<0.05$). Specifically, males swam following significantly more linear trajectories (NGDR=0.13±0.07) than epiphial females (NGDR=0.07±0.02) and parthenogenetic females (NGDR=0.03±0.01). Epiphial females were the most active (A_i=87.2%±3.3%), followed by parthenogenetic females (71.7%±5.2%) and males (60.2%±4.0%).

3.2 Swimming behaviour of *Daphniopsis australis* in the presence of cues

3.2.1 Males

Male swimming speeds significantly differ between treatments (KW test, $P<0.05$). Specifically, experimental males swam significantly faster than control males only in filtered *Isochrysis* and *Daphniopsis* culture water, and in pheromone-conditioned water in the absence and presence of epiphial females (Fig.3a). The other treatments had no effect on male swimming speed, which did not significantly differ ($P>0.05$) from the speed of males swimming in control seawater. Note that males swimming in filtered pheromone-conditioned water swam significantly faster than males swimming in unfiltered pheromone-conditioned water than is in the presence of epiphial females. The presence of parthenogenetic females had no significant effect on male swimming speed. No significant difference between treatments was found (KW test, $P>0.05$) in net-to-gross displacement ratio NGDR (Fig.4a), suggesting that the tortuosity of the trajectories of freely swimming *D. australis* males was independent on the quality of the water. In contrast, the activity index A_i (Fig.3d) significantly differed between treatments (KW test, $P<0.05$); males swimming in filtered and unfiltered *Isochrysis* culture water, in filtered and unfiltered pheromone-conditioned water conditioned with epiphial females, and in filtered *Daphniopsis* culture water were significantly more active than the others, which did not significantly differ from control males.

3.2.2 Epiphial females

The swimming speed of epiphial females (Fig.3b) significantly differed between treatments (KW test, $P<0.01$), and was consistently significantly slower than in the control experiment ($P<0.01$). Specifically, they were significantly faster in filtered and unfiltered *Isochrysis* culture water (6.36±0.26 mm/s) than in both filtered and unfiltered *Daphniopsis* culture water (5.70±0.29 mm/s), and they were the slowest in pheromone-conditioned water either in the presence or the absence of males (4.93±0.40 mm/s). In contrast to the observations conducted on males, NGDR significantly differs between treatments (KW test, $P>0.05$). NGDR only significantly differed, however,

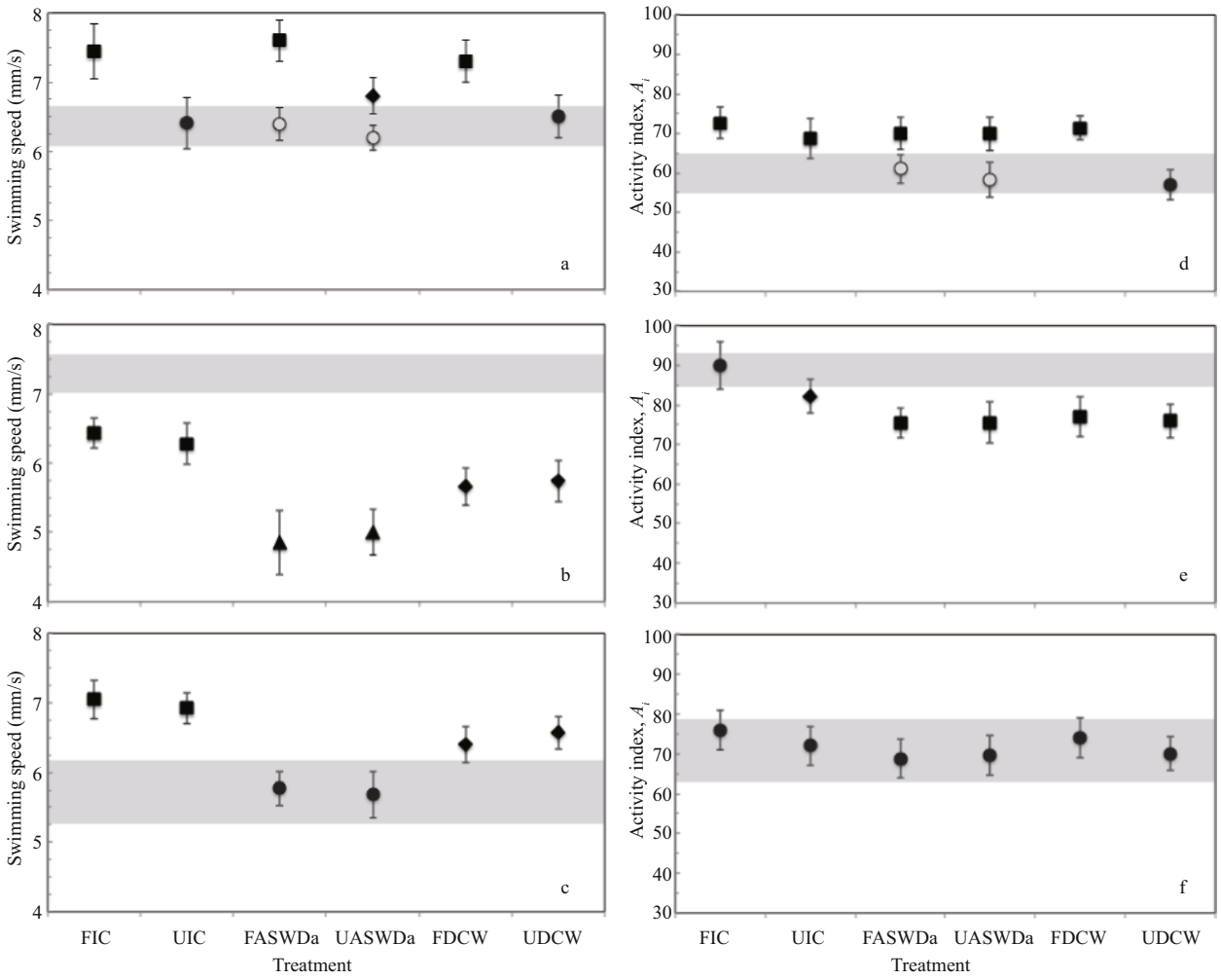

Fig.3 Quantification of the swimming behaviour of male (a, d), epiphial female (b, e) and parthenogenetic female (c, f) of the cladoceran, *Daphniopsis australis* through their swimming speed (a–c) and activity index A_i (d–f) under various conditions of water conditioning

FIC: filtered *Isochrysis* culture; UIC: unfiltered *Isochrysis* culture; FASWDa: filtered artificial seawater conditioned during 24 h with conspecifics of the opposite sex at a concentration of 20 individuals per litre, i.e. partenogenetic females (open symbols) and epiphial females (black symbols) for males (a, d), and males for partenogenetic females (b, e) and epiphial females (c, f); UASWDa: unfiltered artificial seawater conditioned during 24 h with conspecifics of the opposite sex at a concentration of 20 individuals per litre, i.e. partenogenetic females (open symbols) and epiphial females (black symbols) for males (a), and males for partenogenetic females (b) and epiphial females (c); FDCW: filtered *Daphniopsis* culture: UDCW: unfiltered *Daphniopsis* culture. The grey surfaces defined the 95% confidence intervals of swimming speed and NGDR obtained in artificial seawater in the absence of cues, and the error bars represent the 95% confidence intervals. Symbols with different shapes indicate statistical differences significant at the 5% confidence level.

in unfiltered *Isochrysis* water where the tortuosity was significantly lower (i.e. higher NGDR) than in all the other treatments, including the control experiments (Fig.4b). The activity index A_i (Fig.3e) significantly differed between treatments (KW test, $P<0.05$). The activity of epiphial females in filtered *Isochrysis* culture water did not significantly differ from the activity of control females, they were significantly less active in unfiltered *Isochrysis* culture water, where they were significantly more active than filtered and unfiltered male pheromone-conditioned water and filtered and unfiltered *Daphniopsis* culture water, which did not significantly differ from each other.

3.2.3 Parthenogenetic females

The swimming speed of parthenogenetic females (Fig.3c) significantly differed between treatments (KW test, $P<0.05$). Specifically, these females were the slowest in control experiments (5.73±0.40 mm/s) and in pheromone-conditioned water in the presence (5.77±0.25 mm/s) and absence (5.68±0.33 mm/s) of males. Parthenogenetic females were the fastest in filtered *Isochrysis* culture water (7.05±0.28 mm/s) and in unfiltered *Isochrysis* culture water (6.92± 0.22 mm/s), where they swam significantly faster than in filtered *Daphniopsis* culture water (6.40±

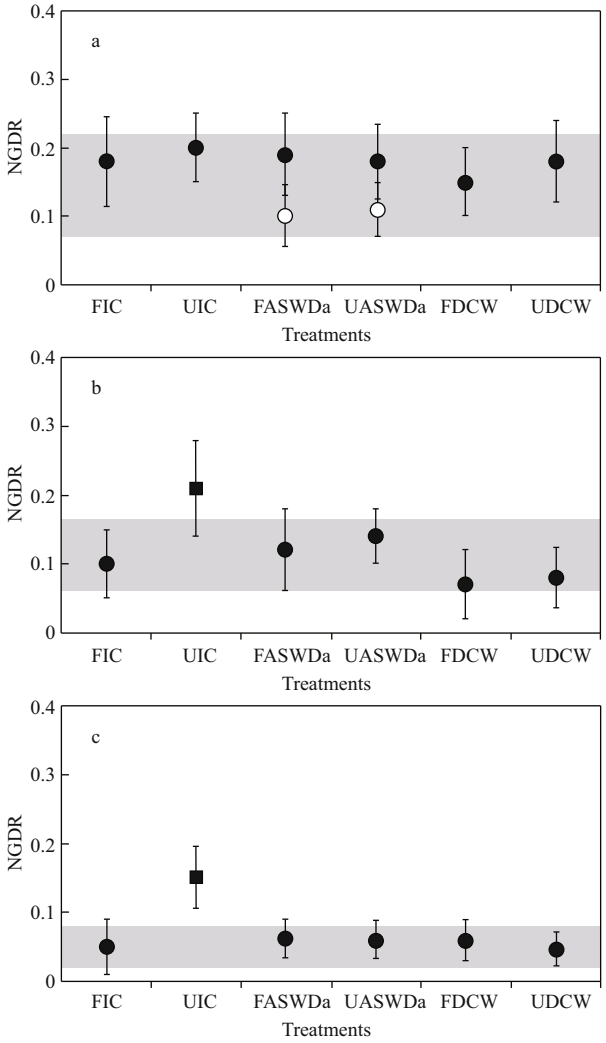

Fig.4 Net-to-gross displacement ratio (NGDR) of the swimming behaviour of males (a), epiphial females (b) and parthenogenetic females (c) of the cladoceran *Daphniopsis australis* under various conditions of water conditioning

FIC: filtered *Isochrisis* culture; UIC: unfiltered *Isochrisis* culture; FASWDa: filtered artificial seawater conditioned during 24 h with conspecifics of the opposite sex at a concentration of 20 individuals per litre, i.e. partenogenetic females (open symbols) and epiphial females (black symbols) for males (a), and males for partenogenetic females (b) and epiphial females (c); UASWDa: unfiltered artificial seawater conditioned during 24 h with conspecifics of the opposite sex at a concentration of 20 individuals per litre, i.e. partenogenetic females (open symbols) and epiphial females (black symbols) for males (a), and males for partenogenetic females (b) and epiphial females (c); FDCW: filtered *Daphniopsis* culture: UDCW: unfiltered *Daphniopsis* culture. The grey surfaces defined the 95% confidence intervals of the NGDR obtained in artificial seawater in the absence of cues, and the error bars represent the 95% confidence intervals.

0.26 mm/s) and in unfiltered *Daphniopsis* culture water (6.57±0.24 mm/s). As reported for epiphial females, NGDR never significantly differ from each other nor from the NGDR obtained in the absence of cues, except for females swimming in unfiltered *Isochrysis* culture water which exhibited significantly less tortuous trajectories (i.e. higher NGDR; Fig.4c). Finally, the activity of parthenogenetic females did not significantly differ between control and experimental treatments (KW test, $P>0.05$).

4 DISCUSSION

4.1 Sex-specific innate swimming behaviour in *D. australis*

In the absence of cues, the adult stages of *D. australis* display a variety of swimming strategies characterised by swimming speeds, trajectory tortuosity and activity levels that are clearly sex-specific (Figs.2 and 3). Specifically, the swimming behaviours of males and females fundamentally differed as the former (Fig.2a) is more space-filling and the related swimming trajectories more rectilinear, while the latter (Fig.2b, 2c) are less space-filling (especially parthenogenetic females; Fig.2c) and characterised by the alternation between short bursts of swimming and sinking phases. Male behaviour is consistent with search models showing that swimming in linear paths and reducing tight turns increases the probability of encountering randomly distributed particles, either food or other individuals (Dusenbery, 1992). Males are then likely to be more tuned towards efficient searching strategies towards food and females, though such a strategy would also increase predation risk. Parthenogenetic females who spend most of their time in close proximity to the bottom (Fig.2c) exhibit a behaviour that is consistent with their biology as it implicitly limits the probability of unneeded encounters with males, while both maximising grazing rates on benthic algae and minimizing the predation risk of laid eggs. The behaviour of sexually reproducing epiphial females then lies somewhere between those of males and parthenogenetic females, and may somehow represent a compromise between optimising encounter rates with males and the need to remain close to the bottom to lay their resting eggs and minimising the related predation risk. These observations further suggest that (i) mate-searching may essentially be a male business, and (ii) males may be filter-feeder, while females (especially parthenogenetic ones) might essentially rely on resources lying on the bottom or in the benthic microlayer. Assessing these hypothesis are, however, far beyond the scope of the present work, and warrant the need for further work on this still seldom known and understood cladoceran species.

4.2 Sex-specific cue hierarchy in *D. australis* swimming behaviour

The responses observed in *D. australis* males and females in the presence of cues from food, conspecifics of the opposite sex, and a mixture of food and conspecific indicate a clear sex-specific cue hierarchy in their swimming behaviour. The implications of these results in the behavioural ecology of *D. australis* are discussed hereafter.

4.2.1 Lack of cue hierarchy in *D. australis* male behaviour

Daphniopsis australis males did not significantly modify the tortuosity of their swimming paths in relation to any of the cues (Fig.4). This result indicates that the available cues did not trigger any change in their search strategies, and contrasts with the widely reported extensive and intensive search strategies expected in food-depleted and food-rich habitats in a variety of organisms ranging from microcrustaceans to megafauna (Seuront and Vincent, 2008; Humphries et al., 2012; Sims et al., 2012; Seuront and Stanley, 2014). This absence of changes in search strategies under various conditions of food and conspecific cues in *D. australis* may suggest a lack of sensory perception in these organisms. However, *D. australis* males significantly increase their activity level in all treatments, except in both filtered and unfiltered male pheromone-conditioned water and in unfiltered *Daphniopsis* culture water (Fig.3d). This result confirms that *D. australis* males are sensitive to chemical cues from both food and conspecifics. More specifically, *D. australis* males significantly increased their swimming speed in filtered *Isochrysis* culture water, filtered *Daphniopsis* culture water and filtered water conditioned with epiphial females. In contrast, their speed did not significantly differ from the speed observed in the absence of cues when they were observed in unfiltered *Isochrysis* culture water, unfiltered *Daphniopsis* culture water and unfiltered water conditioned with epiphial females. These results have several fundamental implications in terms of our understanding of the sensory ecology of *D. australis* males:

1) they have the ability to detect the infochemicals released by phytoplankton cells and conspecifics of the opposite sex. This is consistent with previous observations conducted on the sensory abilities of a variety of microcrustaceans such as cladocerans (Brewer, 1998; La et al., 2014; Nihongi et al., 2016) and copepods (e.g. Woodson et al., 2007; Seuront and Vincent, 2008; Yen et al., 2011; Seuront, 2013; Seuront and Stanley, 2014);

2) they increase their swimming speed in response to chemical exudates of phytoplankton and epiphial females, compared to when exposed to the presence of food and/or epiphial females. This observation is consistent with the widely observed increase in swimming speed in response to physical and chemical cues (Cowles, 2004; Woodson et al., 2007), though exceptions exist (e.g. Seuront, 2013; Seuront and Stanley, 2014). This clear behavioural shift suggests that whilst food and females are actually available males decreases their speed as an active search for food is no longer required, and females may be located through their hydrodynamic trails (Wickramarathna et al., 2014);

3) the lack of significant differences in swimming speed in the presence of cues from phytoplankton exudates (filtered *Isochrysis* culture water), epiphial females exudates and pheromones (filtered female-conditioned water) and a mixture of phytoplankton and conspecifics (filtered *Daphniopsis* culture water) further suggests the absence of cue hierarchy between food and conspecific infochemicals in the sensory ecology of *D. australis* males;

4) finally, the lack of observed behavioural changes when *D. australis* males were swimming in filtered and unfiltered water conditioned with parthenogenetic females further suggest that parthenogenetic females do not generate any infochemicals susceptible to trigger a mate-searching strategy in males or that males have the ability to relate infochemicals to the sexual status of the females that produced them. The slower swimming speed of parthenogenetic females in the absence of cues (and in the presence of filtered male cues; Fig.3c) also suggest that their hydrodynamic trails may be less conspicuous and/or distinct from those of epiphial females. *D. australis* males may hence also have the ability to recognise the sexual status of females based on their hydrodynamic trails.

4.2.2 Cue hierarchy in *D. australis* female behaviour is driven by sexual status

Both epiphial and partenogenetic females shared a lack of significant difference in the observed NGDR between control and experimental treatments, except in unfiltered *Isochrysis* culture water where NGDR significantly increased (Fig.4). This increased tortuosity of their swimming paths indicates a switch towards an intensive search strategy, that is expected in food-rich habitats; see e.g. Seuront and Vincent

(2008), Sims et al. (2012) and Humphries et al. (2012). In contrast, epiphial females consistently decreased their activity level in all treatments, except in filtered *Isochrysis* culture water (Fig.3e), while parthenogenetic females never modified their activity level (Fig.3f).

The response pattern of epiphial and parthenogenetic females swimming speed to experimental treatments further showed clear, though significantly distinct, cue hierarchies that are specific to their sexual status. As observed for males, parthenogenetic females swam significantly faster in *Isochrysis* and *Daphniopsis* culture water than in control experiments (Fig.3c). This indicates an increase in searching activity in the presence of infochemichals originating respectively from food and from a mixture of food and conspecific cues that are in agreement with previous behavioural studies (e.g. Woodson et al., 2007) and the optimal foraging hypothesis (Pyke, 1984). In agreement with their asexual status, parthenogenetic females did not exhibit any change in their swimming speed in male conditioned water (Fig.3c). These results suggest that parthenogenetic females (i) detect the infochemicals released by phytoplankton cells, (ii) do not detect infochemicals from conspecifics, which alternatively may deter their ability to detect phytoplankton infochemicals, and (iii) ignore conspecifics, but consistently actively foraged for food, even when it was readily available as shown by the lack of significant difference in swimming speed between filtered and unfiltered *Isochrysis* culture water and filtered and unfiltered *Daphniopsis* culture water.

In sharp contrast to observations conducted on parthenogenetic females and males, epiphial females consistently decreased their swimming speed compared to control experiments run in the absence of cues (Fig.3b). The intensity of the observed behavioural changes was the smallest in *Isochrysis* culture water, the greatest in male conditioned water, and intermediate in *Daphniopsis* culture water (Fig.3b). These observations suggest that epiphial females (i) have the ability to detect the infochemicals released by both phytoplankton cells and conspecifics of the opposite sex, (ii) hierarchically adjust their behavioural response depending on the nature of the cue, and (iii) prioritise sexual reproduction to feeding as reducing speed is likely to increase the conspicuousness of both their chemical and hydrodynamic trails. This hypothesis is consistent with trail-following experiments showing that *D. daphniopsis* males were more efficient to trail-follow and subsequently capture slow swimming epiphial females (Seuront, unpublished data).

5 CONCLUSION

In the present study, we have shown that *Daphniopsis australis* males, epiphial females, and parthenogenetic females share the ability to detect the infochemicals released by phytoplankton cells and conspecifics of the opposite sex. However, males do not exhibit any hierarchy in their behavioural response to infochemicals from food or conspecifics. In contrast, both epiphial and parthenogenetic females exhibited a clear hierarchy in their behavioural responses to food and conspecific cues. The behaviour of epiphial and parthenogenetic females was then respectively driven by sex-related cues and food-related cues, in agreement with their reproductive status. Note that further behavioural analyses based on the use of robust metrics that are very sensitive to subtle behavioural changes such as fractals and multifractals (Seuront, 2015b) may provide further insights into our understanding of the role of behavioural changes in shaping critical processes such as sexual encounters. In particular, they may allow moving beyond the relatively limiting consideration of mean swimming speed in quantifying encounter rates (Seuront and Stanley, 2014).

More generally, because behavioural changes in microcrustaceans can alter entire biological food webs through e.g. modifications in their trophodynamics, energetics and mating rates (Banse, 1995; Kiørboe, 2008), the results of the present work imply that the understanding of the biology and ecology of cladoceran species, especially in the context of the relatively seldom studied genus *Daphniopsis*, will benefit from detailed investigations of their behavioural properties in response to various biotic cues as food and conspecific infochemicals. This issue is even more critical in an era of global change as Australian inland water bodies which are expected to become drier and more saline. In this context, furthering our understanding of the interplay between temperature and salinity in the resilience of aquatic species to global change may be the key to the successful development and implementation of management and conservation plans.

6 DATA AVAILABILITY STATEMENT

The data used in this work are available upon request to the corresponding author, L. Seuront (laurent.seuront@cnrs.fr).

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