RESEARCH ARTICLE

Influence of flow on locomotion, feeding behaviour and spatial distribution of a suspension-feeding sea cucumber

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ABSTRACT

Although movement in response to environmental conditions represents a fundamental link between animal behaviour and population ecology, it is rarely investigated in suspension feeders because they are generally perceived as sessile. Here, the interplay between water flow and fine locomotor and feeding behaviours was experimentally investigated for the first time in a free-moving suspension-feeding sea cucumber (*Cucumaria frondosa*; Echinodermata, Holothuroidea) using time-lapse videography in a mesocosm setting. Individuals moved away from static conditions in the weakest flow treatment and fled the strongest flows (>40 cm s^{-1}) in the more dynamic treatments. The tentacles of individuals located in areas with flows of ≥40 cm s^{-1} was aligned with the direction of the current, whereas in flows <40 cm s^{-1}, they were typically perpendicular to the direction of flow. Tentacle deployment and insertion rates (i.e. feeding rate) increased with flow, from 0.95 min^{-1} at 10 cm s^{-1} to 1.13 min^{-1} at 40 cm s^{-1}. Three modes of locomotion were detected. Forward crawling was most frequent at flows ≤40 cm s^{-1}, passive rolling dominated at flows >40 cm s^{-1} and active rolling occurred randomly at flows between 0 and 120 cm s^{-1}. Overall, the flow regime favoured by *C. frondosa* was determined to be between 21 and 40 cm s^{-1}, under which an optimal balance between efficient food capture and energy expenditure for attachment to the bottom was presumably found. These findings provide insight into the distribution and population dynamics of suspension-feeding holothuroids, and may also assist the fisheries management and aquaculture development of commercial species.

KEY WORDS: Cucumaria, Benthic animal, Echinoderm, Tentacle insertion rate, Current, Movement

INTRODUCTION

Almost all organisms need to move at some point during their lives, either through active locomotion or through passive transport by physical means (e.g. water, winds) or other agents (Holyoak et al., 2008). The short-term goals of movement mainly relate to reproduction, feeding and survival, and the longer-term goals are to avoid inbreeding and population extinction (Holyoak et al., 2008). The causes, patterns, mechanisms and consequences of movement play an important role in determining the structure and dynamics of populations, communities and ecosystems, as well as the evolution and diversity of life (Holyoak et al., 2008; Nathan et al., 2008).

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Sea cucumbers are echinoderms belonging to class Holothuroidea. They are ubiquitous, sometimes dominant, and they play fundamental roles in marine ecosystems (Purcell et al., 2016). Deposit-feeding sea cucumbers influence the stratification and stability of the sediment via ingestion and bioturbation, and suspension-feeding sea cucumbers modulate water quality by altering its carbonate content and pH (Massin, 1982; Uthicke, 2001). Sea cucumbers are also known to constitute important food sources for other species, such as cod, salmon and walruses (Hamel and Mercier, 2008a, b). Finally, they represent an economically valuable, conservation-worthy marine resource that supports coastal livelihoods around the world (Anderson et al., 2011; Purcell et al., 2013). The habitat requirements, movement patterns, population densities and broad spatial distribution of sea cucumbers have previously been linked to a variety of factors including depth (Mercier et al., 2000a; Woodby et al., 2000), substratum characteristics (Dissanayake and Stefansson, 2012; Hamel and Mercier, 1996; Mercier et al., 2000a,b; Slater and Jeffs, 2010; Woodby et al., 2000; Young and Chia, 1982), food availability (Navarro et al., 2013, 2014; van Dam-Bates et al., 2016), light intensity, temperature and salinity fluctuations (Navarro et al., 2014), as well as predator pressure (Slater and Jeffs, 2010). Under most circumstances, sea cucumbers are considered sedentary or sessile, although they may move in direct response to environmental conditions (Young and Chia, 1982). Substrate type is a well-known driver of distribution; for example, the densities of the deposit-feeding *Parastichopus californicus* in the vicinity of Sitka Sound, Alaska, USA, were highest on shell debris and gravel, and lowest on mud and silt bottoms (Woodby et al., 2000). In a study of another deposit feeder, *Holothuria scabra*, the smallest juveniles (10–40 mm) buried around sunrise and emerged close to sunset, and larger juveniles (40–140 mm) buried when the temperature declined and emerged around mid-day (Mercier et al., 1999). Other deposit-feeding species, such as *Australostichopus mollis* and *Holothuria sanctori*, have been shown to congregate towards areas offering organic-rich food sources (Navarro et al., 2013; Slater and Jeffs, 2010; Slater et al., 2011). Water motion has also been shown to influence distribution in suspension-feeding sea cucumbers, which depend upon currents to bring food particles within reach of their tentacles (Fankboner, 1978; McKenzie, 1991). Clumping in response to wave action was documented along the west coast of South Africa (Barkai, 1991), and dense populations of the dendrochirotid *Aslia lefevrei* were most commonly found in areas of moderately strong water movement (Costelloe and Keegan, 1984a, b).

Cucumaria frondosa (Gunnerus 1767) (Holothuroidea: Dendrochirotida) is the most abundant sea cucumber in the North Atlantic and Arctic Oceans, and it has been the target of commercial fisheries since the 1980s (Hamel and Mercier, 2008b). This long-lived species is distributed along the coast of New England (USA), the eastern coast of Canada, Iceland and Greenland, down

the coast of northern Europe and Scandinavia, as well as in the Faroe Islands (Jordan, 1972). As a suspension feeder, *C. frondosa* ingests suspended particulate food, chiefly phytoplankton, by extending its tentacles in the water column (Hamel and Mercier, 1998). Dense populations are most commonly found on the rocky substrate at depths of ~30 m (Jordan, 1972), but the species occurs from shallow tide pools down to 300–400 m (Klugh, 1924), with few specimens found deeper than 800 m (Hamel and Mercier, 2008b). Fisheries and ecological data have been gathered on *C. frondosa* over the past several decades, including on the reproductive cycle (Hamel and Mercier, 1995), larval development and settlement (Hamel and Mercier, 1996), and feeding (Hamel and Mercier, 1998; Holtz and MacDonald, 2009; Singh et al., 1998, 1999). Knowledge of the spatial distribution patterns and habitat preferences of *C. frondosa* are scant apart from distribution studies conducted in the St Lawrence Estuary (Hamel and Mercier, 1996), which showed that smaller individuals mainly inhabited shallow water (<20 m) and larger ones concentrated in deeper water (≥20 m).

The purpose of the present study was to explore locomotion in this seemingly slow-moving benthic animal and assess how flow influences key behavioural metrics and ultimately determines the spatial distribution of this species. Experiments were first conducted to assess whether the presence or absence of current affected locomotor and feeding behaviours. Another set of experiments was conducted in a large mesocosm to refine our understanding and determine: (i) whether locomotor behaviour and proportions of immobile versus moving individuals vary among different water flows; (ii) whether cloacal respiration and feeding behaviour, indicated by proportions of deployed individuals and tentacle insertion rates (TIRs), are affected by water flows; and (iii) how the ultimate spatial distribution of suspension-feeding sea cucumbers is modulated by water flow. This knowledge will refine our ecological understanding of one of the dominant benthic species in the North Atlantic Ocean, provide tools for more efficient management of suspension-feeding sea cucumber populations, and generate metrics of strategic value for the development of optimal flow conditions during captive breeding and sea ranching.

MATERIALS AND METHODS
Collection, holding and experimental conditions
Approximately 1000 adult sea cucumbers were collected by a fishing vessel (commercially licensed by the federal authority; Fisheries and Oceans Canada) on the southwest Grand Banks of Newfoundland (46°20′43.5″N, 56°23′0.28″W), eastern Canada, at depths between 20 and 30 m. They were kept in a flow-through raceway (11.5×2.5×1.2 m) supplied with 30–60 l min^{-1} of ambient running seawater that was pumped directly from the ocean at 37 m depth. Healthy undamaged sea cucumbers of similar size, with a contracted body length of 15.6±2.5 cm and a wet body mass of 292.5±35.7 g were selected for the experiments. Over the holding and study periods, the water temperature varied seasonally from 1 to 7°C, the salinity was 34–35, the pH was 7.8–8.2, and the dissolved oxygen remained >9.0 mg l^{-1} (all parameters were measured with a YSI® 556 MPS probe). Illumination was provided by multiple fluorescent lights suspended above the holding and experimental tanks. The maximum light intensity was 80–270 lx (Traceable® 3252 light meter) and the light:dark (day–night) cycle varied seasonally from 12 h:12 h to 16 h:8 h light:dark. These ranges of environmental conditions are in line with those occurring in the native habitat of *C. frondosa* (Gianasi et al., 2015). Natural planktonic food (seston biomass: 26.7–34.2 mg l^{-1}) in ambient running seawater was available to sea cucumbers during the study.

Small-scale preliminary experiments
A first set of trials was conducted to verify the assumption that current is a factor in the movement and distribution of *C. frondosa*. They were conducted in two tanks (80×74×26 cm) supplied with ambient seawater and covered by black tarps to prevent interference from external factors; these served as control and experimental tanks. At the onset of a trial, a group of 10 sea cucumbers was placed simultaneously into each of the two tanks, using new individuals for each trial. Over 12 h of acclimation under static conditions, the sea cucumbers moved around freely and distributed themselves randomly in the experimental tanks. Afterward, a small submersible pump (Hydor Koralia Nano 240 aquarium circulation pump) placed at one end of the tank was turned on to generate a nominal bottom current speed of ~27 cm s^{-1} toward the outflow at the other end (the pump in the control tank remained off). This nominal value, which was reduced gradually away from the source, is in the middle of the range determined to be suitable for feeding in *C. frondosa* (Holtz and MacDonald, 2009). Each trial was run for 48 h, after which the individuals were removed and the tanks were drained, cleaned and refilled; the whole process was repeated four consecutive times. To minimize tank effects, the control and experimental tanks were permuted after each replicate. Each trial was recorded via time-lapse cameras sensitive to normal and infrared light (Brinno TLC 200 Pro and MAC 200 DN) set to take one picture of the entire arenas every 10 min and stitch them together into a video output. At night, an LED infrared illuminator (DC 12 V) was automatically turned on as soon as the lights went off to allow recording of nocturnal activity. A grid dividing the tank into six equal rectangles was overlaid on the output videos, and the number of lines crossed by the sea cucumbers was used as an index of horizontal distance travelled along the bottom of the tank over 24 h. The time spent travelling and the time spent with tentacles deployed (an indirect proxy of feeding; Fankboner, 1978; Hamel and Mercier, 1998) over 24 h were determined in the presence and absence of flow.

Large-scale mesocosm experiments
Following the results of the small-scale trials, a set of experiments was conducted in a mesocosm mimicking natural conditions, consisting of a large flow-through raceway (8.25×2.5×0.85 m, length×width×depth). The bottom of the raceway was covered with 20–30 cm of gravel (1–3 cm diameter), with several scattered pebbles and small boulders (8–13 cm diameter). Gravelly and rocky substrates are reported to yield the highest densities of *C. frondosa* (Hamel and Mercier, 1996; So et al., 2010). A plate (5.5 m long) partially divided the tank longitudinally, thus creating a circular flume with unobstructed flow (Fig. 1). The two sections of the raceway on each side of the dividing plate, labeled A and B, were used alternately during the replicate trials to minimize any tank effect. Grid markings were made on the dividing plate and along the raceway, at 50-cm intervals, creating 11 equal areas on each side of the raceway and providing a reference for spatial analysis. The diameters of the inlet and outlet were 4.5 and 10 cm, respectively. Three nominal flow regimes at the water inlet (i.e. maximum flow measured using a handheld Global Water FP211 probe) were used to create three treatments: weakly dynamic (≤20 cm s^{-1}), mildly dynamic (≤100 cm s^{-1}) and highly dynamic (≤200 cm s^{-1}). The three nominal flows generated an increasingly broad continuum of flow regimes across the experimental arena, all consistent with field values (Holtz and MacDonald, 2009). The near-bottom flows (speed and directionality) in the mesocosm were determined by pipetting fluorescein sodium salt (FSA, Sigma-Aldrich) 5 cm from
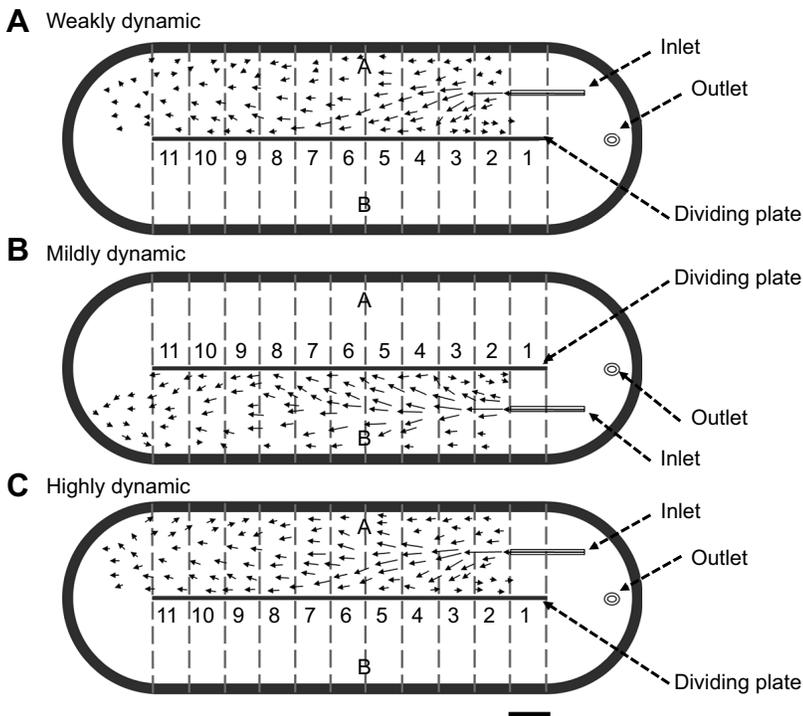
A Weakly dynamic

B Mildly dynamic

C Highly dynamic

Fig. 1. Top view of the experimental raceway (8.25×2.5×0.85 m, length×width×depth) used in the large-scale experiments. (A) Weakly dynamic, (B) mildly dynamic and (C) highly dynamic treatments. The vectors show the direction and strength of the nominal flow in each treatment. The scale bar at the bottom represents 10 cm s^{-1} in A, 50 cm s^{-1} in B and 100 cm s^{-1} in C. The black numbers identify 11 tank sections that provided reference for spatial analysis. Side A or B was used alternately in the replicates of each treatment (shown here alternating across the panels only to illustrate the position of the inlet on both sides).

the bottom and recording the movement of FSA with a camera. As the experimental arena is a raceway, a nearly laminar flow with minor turbulence was generated. The experimental design and the nominal flow regimes for each treatment are illustrated in Fig. 1.

At the beginning of each experiment in the morning (09:00 h), 100 sea cucumbers were evenly spread between areas 2 and 11 at a density of 16 individuals m^{-2} (Fig. 1). After 5 h of acclimation in static conditions, sufficient to allow sea cucumbers to attach to the substrate (Holtz and MacDonald, 2009), the water flow was turned on (14:00 h) (as described above). A time-lapse video camera (described previously) was mounted 3 m above the experimental raceway to render a full view of the arena and allow measurement of the locomotor and feeding behaviours, and spatial distribution of sea cucumbers. To minimize the possibility of environmental effects, the four replicate treatments were conducted alternatingly in side A or B (two replicates for each; Fig. 1). Every replicate run lasted 4 days. No individual was ever used for two successive trials. The water flow in the tank was determined not only in tanks without sea cucumbers (i.e. nominal flows), but also around individual sea cucumbers using the handheld flow probe at the various time points (1, 3, 6, 18, 48 and 96 h) during each replicate run.

Distribution and locomotor behaviour
Based on videos recorded, the distribution of sea cucumbers was determined at various time points (1, 3, 6, 18, 48 and 96 h). The proportion of sea cucumbers positioned under specific flow regimes in each treatment was analyzed over time.

Based on the videos, the proportion of moving individuals in the different flow treatments at various times was calculated as follows: proportion of moving individuals (%)=N_{Mt}/N_T×100, where N_{Mt} is the number of sea cucumbers that moved more than one body length within 1 h prior to time interval t, and N_T is the total number of sea cucumbers. From the above results, only 0–1% of sea cucumbers moved after 6 h in the mildly and highly dynamic treatments. Therefore, the proportions of moving individuals in various sectors of the experimental arena experiencing different flow regimes

the modes of locomotion were compared across treatments at times 1, 3, 6 and 96 h. In addition, the proportion of sea cucumbers in the feeding posture (with tentacles deployed) while using various locomotion modes was determined in the first experimental hour (a universally active phase across treatments).

The proportion of moving individuals in various sectors of the experimental arena experiencing different flow regimes over time was calculated for each treatment as: proportion of moving individuals (%)=N_{Ms}/N_{Ts}×100, where N_{Ms} is the number of sea cucumbers that moved more than one body length within 1 h prior to the specific time intervals in sector s, and N_{Ts} is the total number of sea cucumbers in sector s.

Tentacle deployment and feeding rate
Based on videos, the proportion of sea cucumbers with tentacles deployed (i.e. deployed individuals) at the various time points (1, 3, 6, 18, 48 and 96 h) was calculated for each treatment as: proportion of deployed individuals (%)=N_{Ft}/N_T×100, where N_{Ft} corresponds to the number of sea cucumbers with tentacles fully deployed in the water column at time t, and N_T is the total number of sea cucumbers. In addition, the proportion of deployed individuals in various sectors of the experimental arena experiencing different flow regimes over time was calculated for each treatment as: proportion of deployed individuals (%)=N_{Fs}/N_{Ts}×100, where N_{Fs} corresponds to the number of sea cucumbers with tentacles fully deployed in the water column (i.e. presumably feeding) in sector s at the various times, and N_{Ts} is the total number of sea cucumbers in sector s.

Because the highly dynamic treatment offered the broadest overall range of flow regimes (when considering the distribution of sea cucumbers in the whole mesocosm; see Fig. 1C), the relationship between tentacle insertion rates (TIRs; i.e. feeding rate) and flow speed was studied at the end of this treatment, when the spatial distribution of sea cucumbers had stabilized. TIR was defined as the number of tentacles inserted into the mouth per minute (insertions per minute) and is regarded as a useful indicator of food intake (Holtz and MacDonald, 2009; Singh et al., 1999).

Four feeding sea cucumbers exposed to each of four main flow rates (10, 20, 30 and 40 cm s^{-1}) in each of the treatment replicates were recorded using an underwater camera (GoPro, Hero 4 Silver). The camera was placed close to the focal individual, using an extension pole, to record tentacle movements. Videos were analyzed to determine TIR and the orientation of the crown of oral tentacles relative to current at the various flow speeds. TIRs were determined using frame-by-frame analysis over 10-min intervals. Mean TIRs from a total of 16 individuals for each flow rate were thus obtained.

Cloacal respiration rate

Cloacal movement is regarded as an indicator of respiratory rate, i.e. water circulation in the respiratory tree where oxygenation occurs (Doyle and McNiell, 1964; Gianasi et al., 2015; Jaeckle and Strathmann, 2013). The cloacal movements of sea cucumbers occupying different locations in the tank (submitted to different water flow regimes) were recorded using the GoPro camera at the end of the highly dynamic treatment to determine the variation of respiratory rates in response to different flow rates. This treatment and time point were selected for reasons stated previously. Cloacal respiratory rates were determined by counting the number of cloacal openings over 10-min intervals. Values of openings per minute were averaged for 16 individuals at each flow rate.

Statistical analysis

For the small-scale experiment, the average distance travelled, the time spent travelling and the time spent deployed were compared between the two treatments (presence and absence of flow) using t-tests. For the large-scale experiment, a two-way repeated-measures ANOVA (RM-ANOVA) was used to test the proportion of moving individuals and the proportion of deployed individuals among different time points and flow treatments. When interaction between the factors was significant, each factor was analysed separately using one-way RM-ANOVA, followed by a Bonferroni test. Significant differences between flow rates and the proportion of moving individuals, the proportion of deployed individuals, the locomotion modes, TIRs and cloacal movement were determined using one-way ANOVA followed by *post hoc* multiple comparisons with Tukey's method or t-test. Significance level in all tests was set at $P<0.05$.

RESULTS
Small-scale preliminary experiments

There were significant differences between treatments in both the time sea cucumbers spent travelling ($t=-3.57$, d.f.=14, $P=0.003$) and the distance they travelled ($t=-4.56$, d.f.=14, $P<0.001$) over 24 h, which both were less in the presence of flow (mean of 65 min and 91 cm, respectively) than in its absence (mean of 97 min and 140 cm; Fig. 2A,B). Inversely, the time spent wirth tentacles deployed over 24 h was significantly greater in the presence of flow (10 h) than in its absence (3 h) ($t=11.42$, d.f.=14, $P<0.001$; Fig. 2C).

Large-scale experiments
Distribution and locomotor behaviour

Overall, marked flow preferences were determined and no aggregation behaviour was detected. The sea cucumbers typically spread out in the experimental arena under all flows tested. They moved away from near-zero flow conditions over time under weak flow, whereas under mildly and highly dynamic flow, individuals consistently moved away from the strongest flow regimes >40 cm s^{-1}.
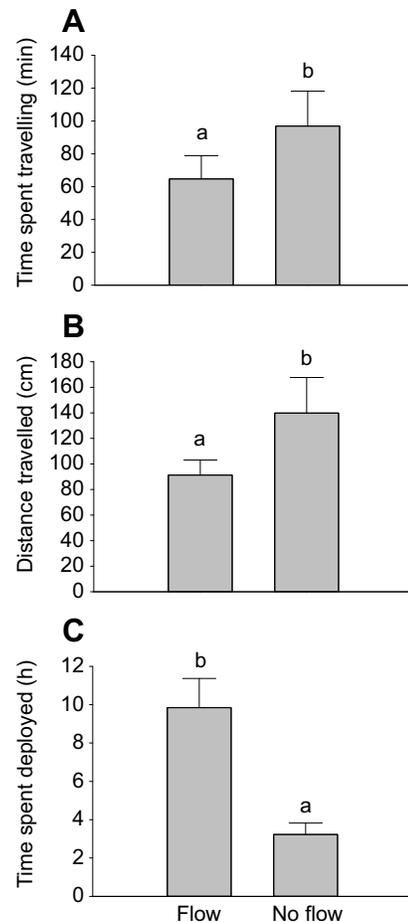

Fig. 2. Feeding and locomotion behaviour of *C. frondosa* with and without flow. Effect of the presence/absence of flow on (A) time spent travelling, (B) horizontal distance travelled and (C) time spent with tentacles deployed over 24 h. Data are shown as means±s.d. for 10 individuals in each treatment (*n*=4 replicate runs per treatment). Means with different letters are significantly different (*t*-test, *P*<0.05).

Specifically, in the weakly dynamic treatment, the proportion of sea cucumbers located in areas with flows of <10 cm s^{-1} decreased from 91% at the beginning of the experiment (0 h) to 80% at the end of the experiment (96 h), whereas the proportion of sea cucumbers located in areas with flows of 10–20 cm s^{-1} increased from 9 to 20% (Fig. 3A). In the mildly dynamic treatment, the proportion of sea cucumbers located in areas with flows of >80 cm s^{-1} decreased from 7 to 0% after only 3 h, and no sea cucumbers were found in areas with flows >60 cm s^{-1} after 6 h (Fig. 3B). In the highly dynamic treatment, the proportion of sea cucumbers located in areas with the strongest flows (>120 cm s^{-1}) decreased from 10 to 0% after only 1 h, whereas the proportion of sea cucumbers located in areas with the lowest flows (≤20 cm s^{-1}) increased from 41 to 63%. No sea cucumbers were found in areas with flows >80 cm s^{-1} after 6 h in the highly dynamic treatment (Fig. 3C). At the end of the mildly and highly dynamic treatments (96 h), no sea cucumbers were found in areas with flows >40 cm s^{-1}.

A two-way RM-ANOVA showed that both treatment ($F_{2,6}=43.44$, $P<0.001$) and time ($F_{5,15}=116.78$, $P<0.001$) had a significant influence on the proportion of moving sea cucumbers, but also revealed a significant interaction between the two factors ($F_{10,30}=19.746$, $P<0.001$; Fig. 4). Independent analyses at each level (one-way RM-ANOVA) showed that the proportion of moving sea
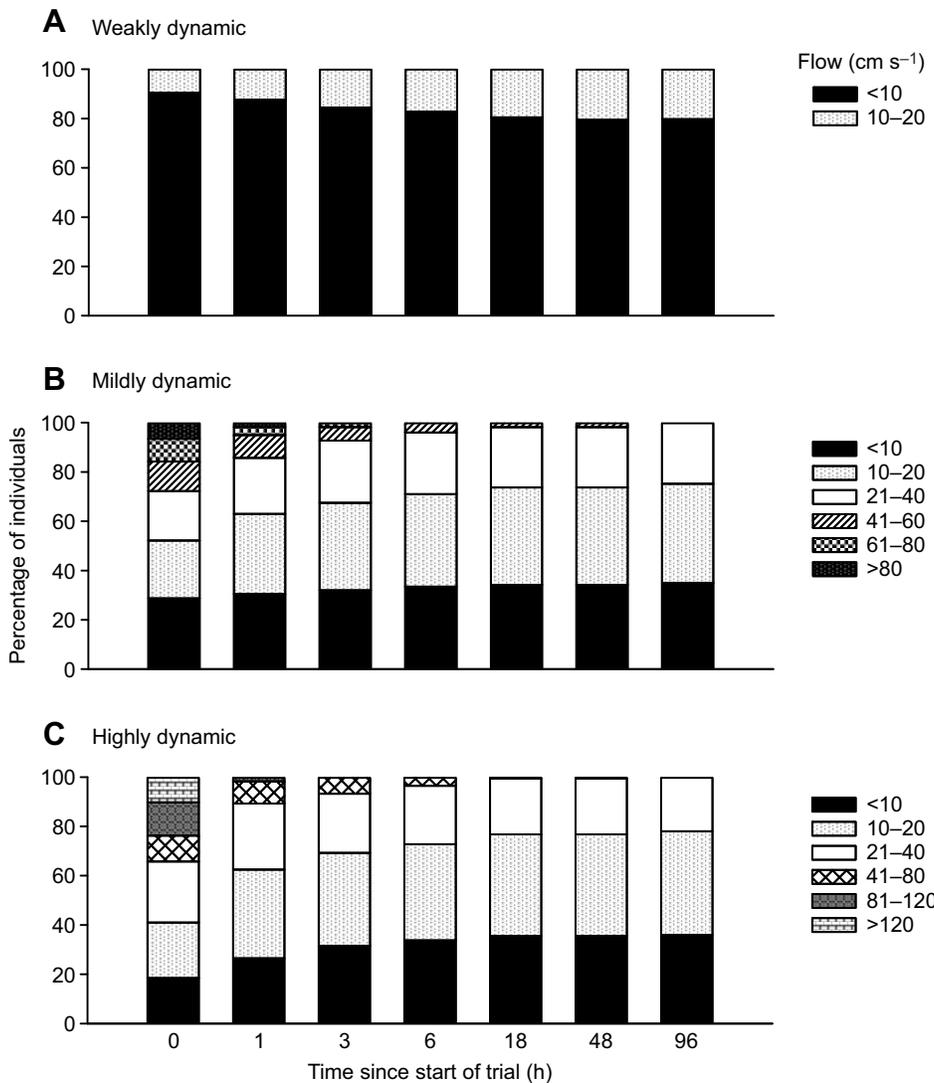
Fig. 3. Distribution of 100 individuals of *C. frondosa* in various sectors of the experimental arena experiencing different flow regimes over time (0, 1, 3, 6, 18, 48 and 96 h). (A) Weakly dynamic, (B) mildly dynamic and (C) highly dynamic treatments. Data are shown as means (*n*=4).

cucumbers was significantly affected by treatment at all time points (Table S1). During the first experimental hour, the proportion of moving sea cucumbers in the highly dynamic treatment was significantly higher than in the weakly and highly dynamic treatments (Bonferroni test, $P<0.05$). From the third hour (3 h) to the end of the experiment (96 h), the proportion of moving sea cucumbers in the weakly dynamic treatment was significantly higher than in the two other treatments ($P<0.05$), and no significant differences were found between the mildly and highly dynamic

treatments ($P>0.05$). No significant differences in the proportion of moving individuals were found across time points in the weakly dynamic treatment ($F_{5,15}=1.07$, $P=0.415$), but significant differences over time were found in the mildly ($F_{5,15}=35.95$, $P<0.001$) and highly dynamic treatments ($F_{5,15}=78.93$, $P<0.001$). Specifically, the proportion of moving individuals was significantly greater at 1 h than at any other time in both treatments (Bonferroni test, $P<0.05$), but no significant differences were found among 6, 18, 48 and 96 h ($P>0.05$).
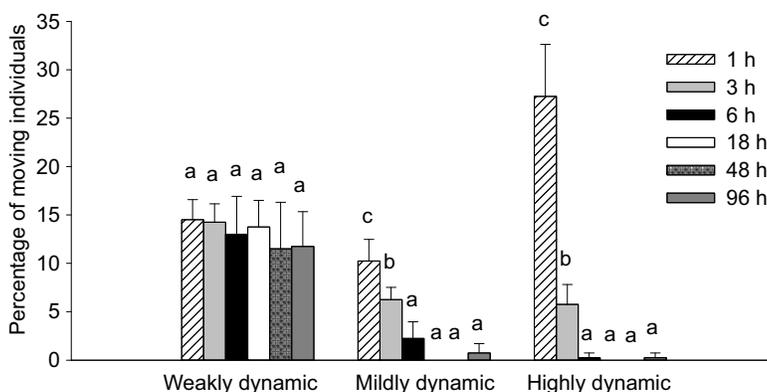

Fig. 4. Percentage of individuals of *C. frondosa* that were scored as moving at the different time points (1, 3, 6, 18, 48 and 96 h) under the three treatments (weakly, mildly and highly dynamic environments). Data are shown as means±s.d. for 100 individuals in each treatment (*n*=4 replicate runs per treatment). Different letters highlight significant differences between different time points within treatments (two-way RM-ANOVA, $P<0.05$).
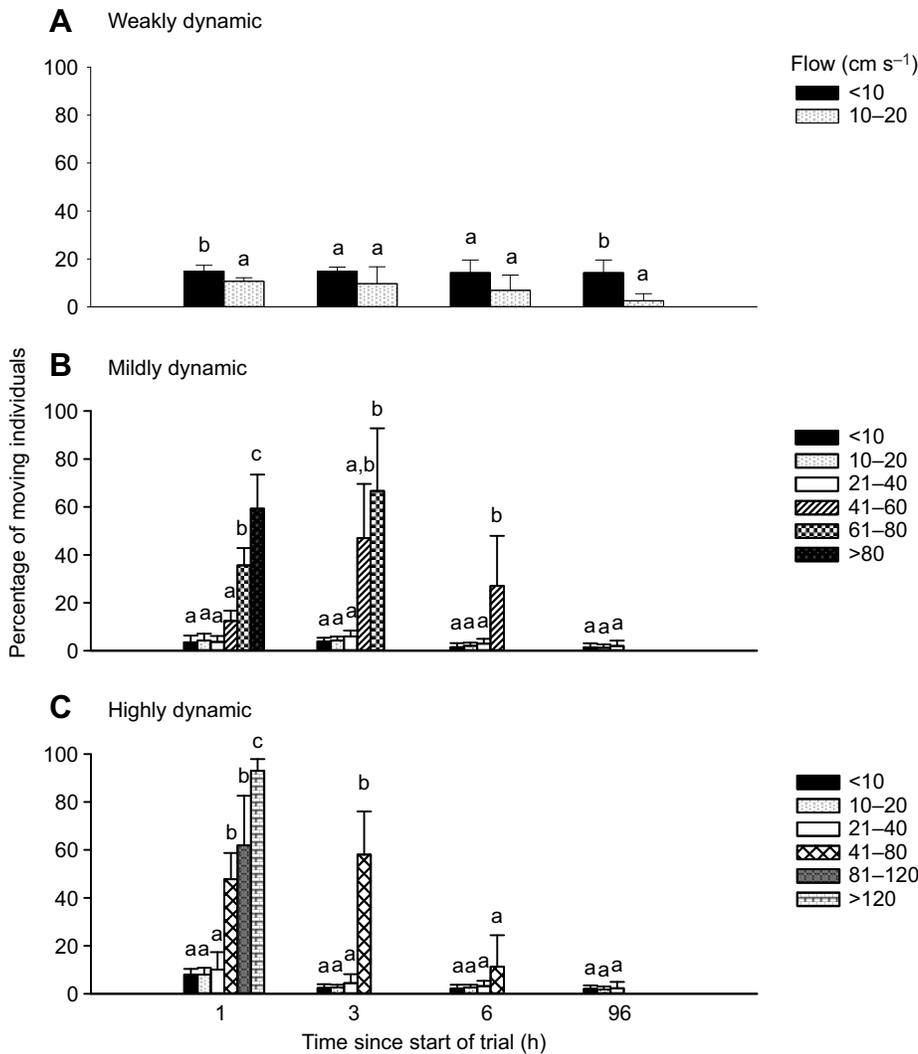
Fig. 5. Percentage of individuals of *C. frondosa* that were scored as moving in various sectors of the experimental arena experiencing different flow regimes over time (1, 3, 6 and 96 h). (A) Weakly dynamic, (B) mildly dynamic and (C) highly dynamic treatments. Data are shown as means±s.d. (*n*=4). Means with different letters are significantly different (ANOVA or *t*-test, *P*<0.05).

In the weakly dynamic treatment, the proportion of moving sea cucumbers located in more dynamic areas (flows of 10–20 cm s^{-1}) was significant greater than in areas with flows of <10 cm s^{-1} at 1 and 96 h (Table S2; Fig. 5A). In the mildly dynamic treatment, the proportion of moving sea cucumbers was significantly affected by flow regimes at all time points except 96 h (Table S2; Fig. 5B). During the first hour of the mildly dynamic treatment, the proportion of moving sea cucumbers located in areas with flows of 61–80 and >80 cm s^{-1} were significant greater than in other areas (Tukey's test, *P*<0.05; Fig. 5B). In the highly dynamic treatment, the proportion of moving individuals was also significantly affected by flow regimes at 1 and 3 h (Table S2; Fig. 5C). Movements at other times points were nearly null. During the first hour of the highly dynamic treatment, the proportion of moving sea cucumbers located in areas with flows of 41–80, 81–120 and >120 cm s^{-1} were significant greater than in other areas (Tukey's test, *P*<0.05; Fig. 5C).

Tentacle deployment and feeding rate

A two-way RM-ANOVA showed that both treatment ($F_{2,6}$=42.22, *P*<0.001) and time ($F_{5,15}$=75.07, *P*<0.001) had a significant influence on the proportion of deployed sea cucumbers, but also revealed a significant interaction between the two factors ($F_{10,30}$=10.52, *P*<0.001; Fig. 6). The results of independent one-way RM-ANOVA showed that the proportion of deployed sea

cucumbers was significantly affected by treatment at all time points (Table S3). From the beginning to the end of the experiment, the proportion of deployed sea cucumbers in the weakly dynamic treatment remained significantly lower than in the mildly and highly dynamic treatments (Bonferroni test, *P*<0.05). In addition, the proportion of deployed sea cucumbers varied significantly over time under all treatments (weakly dynamic, $F_{5,15}$=10.80, *P*<0.001; mildly dynamic, $F_{5,15}$=31.26, *P*<0.001; highly dynamic, $F_{5,15}$=44.84, *P*<0.001). The proportion of sea cucumbers with tentacles deployed at 1 h was significantly lower than at all other times in all treatments (Bonferroni test, *P*<0.05), and no significant differences were found among 6, 18, 48 and 96 h in all treatments (*P*>0.05).

In the weakly dynamic treatment, the proportion of deployed sea cucumbers located in areas with flows of 0–10 cm s^{-1} was significantly lower than in areas with flows of 10–20 cm s^{-1} at all time points (Table S4; Fig. 7A). Only 11–18% of sea cucumbers located in areas with flows of <10 cm s^{-1} had tentacles deployed at all time points, compared with 49–60% of sea cucumbers located in areas with flows of 10-20 cm s^{-1}. In the mildly-dynamic treatment, the proportion of deployed sea cucumbers was significantly affected by flow regimes at all time points except at 1 h (Table S4; Fig. 7B). The proportion of deployed sea cucumbers located in areas with flows of 21–40 cm s^{-1} was highest (31–85%) at all time points (Fig. 7B). In the highly dynamic treatment, the proportion of
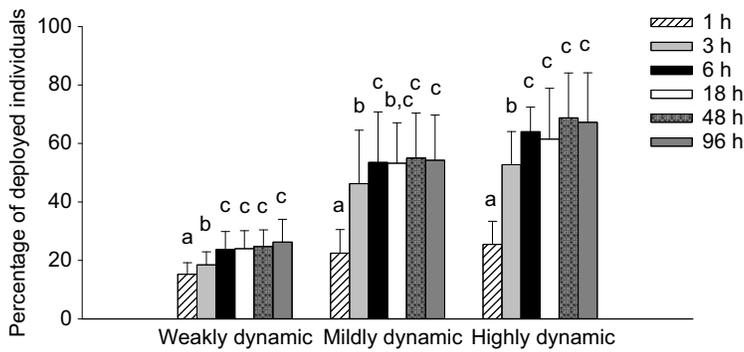
Fig. 6. Percentage of individuals of *C. frondosa* with tentacles deployed at various time points (1, 3, 6, 18, 48 and 96 h) under different treatments (weakly, mildly and highly dynamic environments). Data are shown as means±s.d. for 100 individuals in each treatment (*n*=4 replicate runs per treatment). Means with different letters show significant differences between time points within treatments (two-way RM-ANOVA, *P*<0.05).

deployed sea cucumbers was significantly affected by flow regimes at all time points (Table S4; Fig. 7C). The proportion of deployed sea cucumbers located in areas with flows of 81–120 cm s⁻¹ was lowest at 1 h (13±10%), and the proportion of deployed sea cucumbers located in areas with flows of 21–40 cm s⁻¹ was highest (49–90%) at all time points (Fig. 7C).

TIRs varied significantly among sea cucumbers positioned in different flow regimes across the experimental arena ($F_{3,60}$=15.50, *P*<0.001, Fig. 8A). TIRs increased from a low of 0.95±0.09 insertions min⁻¹ at flows of 10 cm s⁻¹ to a high of 1.13±0.06 insertions min⁻¹ at flows of 40 cm s⁻¹ (Fig. 8A).

Cloacal respiration rate

Cloacal movements measured during the highly dynamic treatment did not differ significantly among sea cucumbers positioned in

different flow regimes across the experimental arena ($F_{3,60}$=0.79, *P*=0.503; Fig. 8B). Nevertheless, individuals located in stronger flow regimes generally had higher respiration rates, which ranged from 1.95±0.25 openings min⁻¹ at a flow regime of 10 cm s⁻¹ to 2.09±0.27 openings min⁻¹ at 40 cm s⁻¹.

Behavioural observations

Tentacle orientation

Adults of *C. frondosa* possess 10 oral tentacles, and they feed using a sequence of coordinated movements: one of the tentacles, bearing food materials, is slowly folded into the oral cavity, and is then outstretched again as another tentacle is being introduced in the mouth. The same action is repeated continuously during the feeding period.

Videos and still pictures revealed that the crown of tentacle of the sea cucumbers had different orientations under different water flow
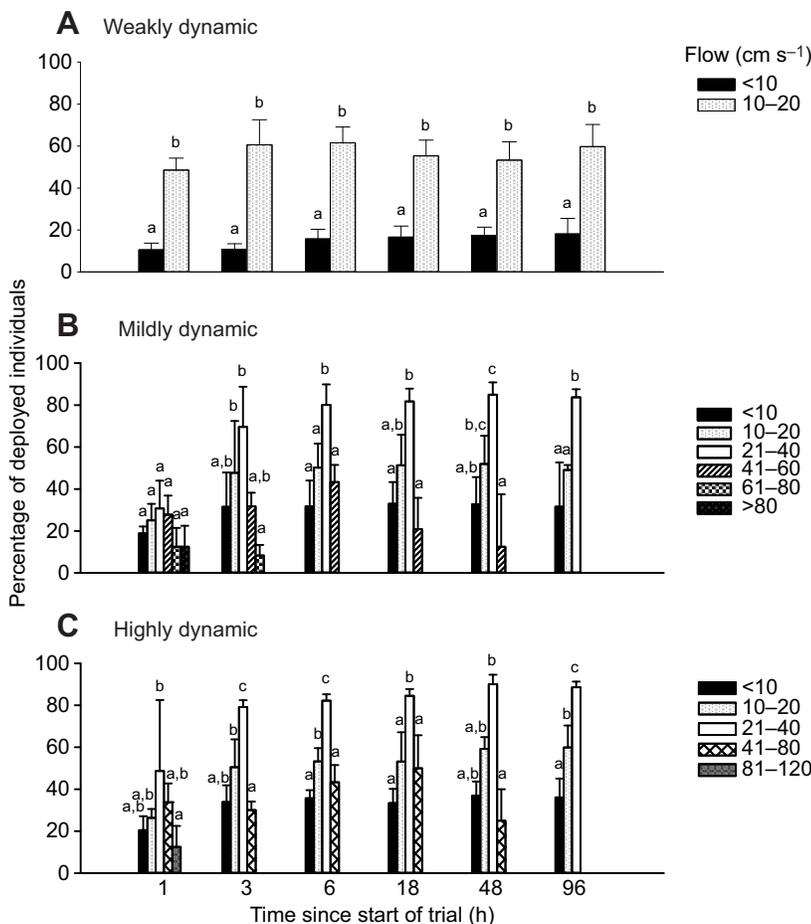

Fig. 7. Percentage of individuals of *C. frondosa* with tentacles deployed in various sectors of the experimental arena experiencing different flow regimes (from 0–10 to 120–200 cm s⁻¹) over time. (A) Weakly dynamic, (B) mildly dynamic and (C) highly dynamic treatments. Data are shown as means±s.d. (*n*=4). Means with different letters are significantly different (one-way ANOVA or *t*-test, *P*<0.05).
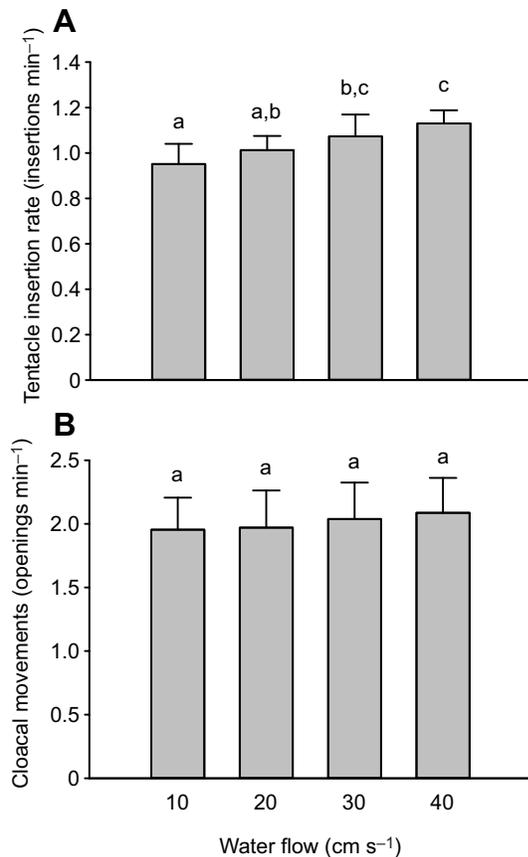
Fig. 8. Effect of flow on the feeding and cloacal (respirator) behaviour of *C. frondosa*. Tentacle insertion rates (A) and cloacal movements (B) under different flow regimes. Data are shown as means±s.d. (*n*=16 individuals per flow regime). Means with different letters are significantly different (ANOVA, *P*<0.05).

Fig. 9. Orientation of the crown of tentacles of *C. frondosa* under different flow regimes. The arrow shows the direction of the flow and its length is proportional to the flow speed. (A) The crown of tentacles follows the direction of the flow at $\geq$40 cm s^{-1}. (B) The crown of tentacles directly faces the direction of the flow at 20 cm s^{-1}. (C) The crown of tentacles is perpendicular to the direction of the flow at 10 cm s^{-1}. (D) The crown of tentacles is perpendicular to the direction of the flow at <10 cm s^{-1}.

regimes. The crown of tentacles of individuals located in areas with flows $\geq$40 cm s^{-1} tended to follow the direction of the current and was not fully deployed (Fig. 9A). The crown of tentacles of individuals located in areas with flows between 10 and 40 cm s^{-1} typically faced the current or was perpendicular to the direction of water flow (Fig. 9B,C). The crown of tentacles of individuals located in areas with flows <10 cm s^{-1} was typically perpendicular to the direction of water flow (Fig. 9D). Overall, tentacles were only fully deployed at flows between 0 and 40 cm s^{-1} (Fig. 9B–D).

Locomotion modes

Three different modes of locomotion were detected; namely, forward crawling (moving forward through contraction–extension and using podia on ventral surface; Movie 1), active rolling (on the longest body axis without significant body shape change; Movie 2) and passive rolling (carried by water movement; Movie 3). Overall, 42±10% of sea cucumbers that used crawling, 25±19% that used active rolling and 10±6% that used passive rolling were found to extend their tentacles when moving. The movement speed of forward crawling and active rolling was similar, ranging from 0.2 to 0.9 m h^{-1}. However, the movement speed of passive rolling was 180–3000 m h^{-1}, which mainly depended on the water flow rate.

In the weakly dynamic treatment of the large-scale experiments, 68–85% of moving sea cucumbers located in areas with flows of

<10 cm s^{-1} were crawling, and 15–32% used active rolling or alternated crawling and active rolling at all time points. Between 38 and 75% of moving sea cucumbers located in areas with flows of 10–20 cm s^{-1} were crawling, and 25–62% used active rolling or alternated between crawling and active rolling at all time points. During the first experimental hour of the mildly dynamic treatment, no sea cucumbers located in areas with flows of $\leq$40 cm s^{-1} used passive rolling, and 75–81% displayed forward crawling. However, 84±16% of moving sea cucumbers located in areas with flows of >80 cm s^{-1} used passive rolling, and only 5±4% displayed forward crawling. During the third experimental hour of the mildly dynamic treatment, 58–75% of moving sea cucumbers located in areas with flows of 41–80 cm s^{-1} displayed passive rolling. During the first experimental hour of the highly dynamic treatment, no sea cucumbers located in areas with flows of <40 cm s^{-1} displayed passive rolling, and 60–71% of them exhibited forward crawling. Inversely, 64–93% of moving sea cucumbers located in areas with flows of >80 cm s^{-1} displayed passive rolling, and only 2–6% displayed forward crawling.

DISCUSSION

Forces imposed by moving water can dramatically influence the locomotor behaviour of marine organisms (Wildish and Kristmanson, 2005). Behavioural responses to flow include rheotaxis, which is a directed response to flow direction involving locomotion or muscular turning of body parts, and rheokinesis, which is a non-directed response causing random movement proportional to flow velocity (Wildish and Kristmanson, 2005). Relatively few suspension feeders have significant locomotor capabilities because most of them are sessile or tube-dwelling. Therefore, previous studies on suspension feeders in response to flow mainly focused on their feeding behaviour and body/appendage orientation. For sea cucumbers, most behavioural studies have been conducted on deposit-feeding species, and food availability was identified as the main factor driving movement

(Navarro et al., 2014; Slater et al., 2011; van Dam-Bates et al., 2016). In the present study, water flow was shown to play an important role in eliciting displacement in *C. frondosa*, a free-moving suspension feeder. Individuals tended to move away from near-zero flow conditions over time and avoid the strongest flow areas >40 cm s^{-1}. It can be suggested that in weakly dynamic environments, *C. frondosa* sought stronger flows to find more suitable grounds for passive suspension feeding (i.e. conditions likely to supply phytoplankton and other suspended particles). However, in more dynamic environments, *C. frondosa* fled flow regimes above a certain strength, presumably to minimize drag that would impede suspension feeding and might induce dislodgement. Pan et al. (2015) observed that the sea cucumber *Apostichopus japonicus*, which feeds on deposited organic matter, moved downstream under high flows of 30 cm s^{-1}, and moved either downstream or upstream under flows of 15 cm s^{-1}. For deposit feeders, displacement from dynamic to weaker flows is chiefly about reducing drag effects. For suspension feeders such as *C. frondosa*, it is also, and perhaps more importantly, a question of capture/feeding efficiency.

Water flow was determined to drive not only the movement but also the activity level of *C. frondosa*. In the small-scale experiments conducted under weak flow, the time spent travelling and the distance travelled were less in the presence of flow than in its absence. Similarly, in the large-scale mesocosm experiments, 12–15% of the sea cucumbers kept moving throughout the 96 h of the trial in the weakly dynamic treatment, suggesting that the available flow regimes were not deemed suitable or optimal. Conversely, few sea cucumbers were moving after 6 h in the mildly and highly dynamic treatments, suggesting they had found suitable flow conditions within that interval. This finding contrasts with the locomotion of deposit feeders such as *A. japonicus*, in which water current consistently acts as a stimulating factor, causing an increase in movement (Pan et al., 2015). It emerges that *C. frondosa* can actively move away from suboptimal flow regimes (that are either too weak or too strong) and towards more suitable intermediate conditions (10–40 cm s^{-1}). Another study conducted on a suspension feeder, the polychaete *Manayunkia speciosa*, showed that it moved from high flow to low flow to increase survivorship (Malakauskas et al., 2013).

An unexpected finding of the present study was that *C. frondosa* exhibits different modes of locomotion, i.e. not only the typical forward crawling, but also active rolling (on the side) and passive rolling, in response to different flow regimes. Forward crawling remained the most frequently observed locomotor behaviour under weak flow regimes. It also commonly occurred in areas with flows <40 cm s^{-1} in the more dynamic treatments, suggesting that it is widely used by *C. frondosa* when flow conditions are below that threshold (40 cm s^{-1}). Low incidence of active rolling (moving sideways while remaining partly attached to the bottom) consistently occurred under all the flows tested up to 120 cm s^{-1}, whereas passive rolling occurred strictly in areas with flows >40 cm s^{-1}. There are two main differences between these two modes of locomotion. First, passive rolling relies partly on the power of flow, whereas active rolling is independent of flow. Second, passive rolling involves a change of shape (ballooning) to increase buoyancy (J.-F. Hamel, J. Sun, B. L. Gianasi, E. M. Montgomery, E. L. R. Kenchington, B. Burel, P. Winger, S. Rowe and A. Mercier, unpublished data), whereas active rolling does not. In the highly dynamic treatment, the proportion of sea cucumbers located in areas with the lowest flows (≤20 cm s^{-1}) increased from 41 to 63%. Based on the video records, it appears that sea

cucumbers initially located in flows >40 cm s^{-1} were passively carried (rolling) to the opposite area. However, sea cucumbers in the weakest flows actively crawled towards areas with flows between 21 and 40 cm s^{-1}, suggesting relocation to a preferred habitat. In other words, passive rolling seems to be used by sea cucumbers to move from strong to weak flow areas, after which they may move by crawling or active rolling towards definitive optimal locations. This diversity in locomotor behaviour is currently being investigated, as it would suggest that sea cucumbers can move much faster and over greater distances than typically assumed (Hamel et al. submitted), and not only during escape responses to a predator (Margolin, 1976). These modes of locomotion may enhance their responsiveness to environmental stimulation and favour broader distribution ranges and massive relocation when required, even at the adult stage.

The present study highlighted a close relationship between water flow and feeding in *C. frondosa*, based on tentacle deployment, orientation and insertion rates. Under flows <20 cm s^{-1}, the crown of tentacles typically faced the current, which may help *C. frondosa* capture more food particles per unit of time (increase efficiency). More typically sessile suspension-feeding benthic organisms, such as scallops (Sakurai and Seto, 2000), sea fans (Leversee, 1976), sea anemones (Anthony, 1997) and crinoids (Leonard et al., 1988), also orient themselves or their feeding appendages relative to currents in order to enhance their capture capacities and exploit their food supply optimally. Here, in flows ≥40 cm s^{-1}, *C. frondosa* oriented its tentacles to follow the direction of the current and did not deploy them fully, which was reported in a previous study (Holtz and MacDonald, 2009), likely to alleviate the deformation of the tentacles caused by the increase in flow. Changes in orientation in response to variable water currents has also been observed in a few elasipodid species, including *Peniagone japonica* distributed in deep bays in Japan (Okada and Ohta, 1993). Moreover, most individuals of the species *Irpa abyssicola* filmed on the bottom of Hayes Deep (2700 m depth, Norwegian and Greenland Seas) faced into the current, whereas on the slope they mainly oriented with the current (Gebruk et al., 2003). To a large extent, tentacle deployment in *C. frondosa* can be associated with feeding behaviour, although the tentacles may be extended without any movements towards the mouth for several hours (Hamel and Mercier, 1998), indicating that it serves other purposes as well. It is possible that *C. frondosa* uses its tentacles to detect the direction and strength of water flow and reacts accordingly by adjusting its body orientation or by moving. Optimal location and orientation relative to flow presumably allow this planktivorous species to maximize food capture and minimize energetic costs.

Cucumaria frondosa, like other passive suspension feeders, depends entirely on the ambient flow to supply food particles to its feeding appendages (Leichter and Witman, 1997; Singh et al., 1998). This may explain why the time spent with tentacles deployed in *C. frondosa* was greater in the presence of flow than in the absence of flow. However, in the mildly and highly dynamic treatments, the proportion of sea cucumbers with tentacles deployed was relatively low (8–12%) especially in areas with flows >80 cm s^{-1}. This may be related to an increasing difficulty in deploying tentacles. The cessation of feeding (tentacle retraction) by dendrochirotids in strong currents may occur to avoid damage or may be due to a failure to maintain efficient particle capture (McKenzie, 1987). In the two more dynamic treatments, the proportion of deployed sea cucumbers located in areas with flows of 21–40 cm s^{-1} was greater than in all other areas (with different flows) at given time points, suggesting that this optimal range allows

C. frondosa to fully deploy its tentacles while providing a sufficient supply of particulate food.

Beyond the proportion of individuals with deployed tentacles, the TIR is a more specific indicator of feeding activity (Holtz and MacDonald, 2009; Singh et al., 1999). Holtz and MacDonald (2009) used a small laboratory flume holding five individuals to determine that TIRs were not affected by water flows <40 cm s^{-1} and were reduced when flow was above a threshold velocity of ~55 cm s^{-1}. In contrast, the present study found that TIRs increased with increasing water flow between 0 and 40 cm s^{-1} in the larger life-size tank setup, which held 100 individuals and may have provided *C. frondosa* with an environment that is closer to natural conditions. As the water flow increases, suspension feeders will more easily capture food particles because more particles come into contact with their feeding structures (Shimeta and Jumars, 1991). This could mean that *C. frondosa* needs less time to capture food particles before the tentacles are inserted into the mouth with increasing water flow, up to a certain threshold, as discussed previously. From results obtained here combining the proportion of deployed sea cucumbers and TIR, the optimum water flow for *C. frondosa* to feed lies between 21 and 40 cm s^{-1}.

Sea cucumbers respire mainly by two mechanisms: one is by taking up oxygen across the general body surface (Hopcroft et al., 1985), and the other is by drawing and expelling water through the cloaca, in and out of the respiratory tree (Woodby et al., 2000). In the present study, cloacal movement (respiration rate) was not related to the water flow. In contrast, the need for more frequent renewal of water in the respiratory tree is a good indicator of stress in sea cucumbers (Gianasi et al., 2015; Shiell, 2006). Therefore, experimental results of the present study have shown that *C. frondosa* was stress-free at flows ≤40 cm s^{-1} and was otherwise well adapted to a wide range of water flows.

Ultimately, the final spatial distribution displayed by *C. frondosa* in the mesocosm reflected the fact that, as a passive suspension feeder, it must seek optimal water flow to subsist. In areas where the water flow was zero or close to zero, a proportion of sea cucumbers was still moving around even after 4 days, presumably in search of better conditions. In contrast, strong flow may impede tentacle deployment in *C. frondosa*, as outlined previously, and it also requires more energy to adhere to the bottom as suggested in *Apostichopus japonicus* (Pan et al., 2015). Here, between 50 and 93% of sea cucumbers were moving during the first hour spent in the mildly and highly dynamic treatments, which generated locally high flow regimes of >80 and >120 cm s^{-1}, respectively. After 96 h, no sea cucumbers were found in areas with flows >40 cm s^{-1}. However, a previous study revealed that *C. frondosa* can colonize areas of water flow >40 cm s^{-1} in the field (Holtz and MacDonald, 2009). There are a number of possible explanations for this. The high water velocity in the field may have been transient; for example, the sea cucumbers at Jamieson Island were exposed to high velocities of 99–130 cm s^{-1} only 17% of the time (Holtz and MacDonald, 2009). Flow velocities in the field are more variable than in the laboratory, changing with tides and oceanographic conditions (e.g. storms), and the seafloor is more complex (rocks, crevices, macrophytes), offering zones with moderate current even under high flows. Furthermore, gregarious or clumping behaviour in the wild can enhance the resistance of organisms to dynamic environments, as exemplified by a study in the sea cucumbers *Thyone aurea* and *Pentacta doliolum* on the west coast of South Africa (Barkai, 1991).

In conclusion, the present study highlighted significant shifts in the locomotor and feeding behaviours of a suspension-feeding sea cucumber in response to water flow. The findings not only provide novel information on the ecology of suspension feeders, but will help our understanding of the broad yet patchy distribution of sea cucumbers in various environments, and will be of strategic value to the management of commercial fisheries. In addition, knowledge of the preferred conditions of water flow in suspension-feeding sea cucumbers will inform the design of holding conditions in the context of captive breeding, integrated multi-trophic aquaculture and sea ranching.

Author contributions
Conceptualization: J.S., J.-F.H., A.M.; Methodology: J.S., J.-F.H., A.M.; Formal analysis: J.S.; Investigation: J.S.; Writing - original draft: J.S.; Writing - review & editing: J.S., J.-F.H., A.M.; Supervision: J.-F.H., A.M.; Project administration: A.M.; Funding acquisition: J.S., A.M.

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