

Contribution to the Theme Section 'Jellyfish bloom research: advances and challenges'

# Swimming behaviour can maintain localised jellyfish (*Chironex fleckeri*: Cubozoa) populations

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**ABSTRACT:** The potentially lethal *Chironex fleckeri* (Class Cubozoa) inhabits estuarine and near-shore coastal waters in the western Pacific. The spatial scales of connectivity between *C. fleckeri* populations are poorly understood. Biophysical modelling of Port Musgrave, a 17 × 21 km shallow bay in tropical Australia, was used to investigate the potential for connections between populations separated by medium (10s of km) to small (100s of m) spatial scales. We measured the swimming speeds and orientations of medusae ranging in size from 4 to 12 cm interpedalial distance (the distance between two adjacent corners on the bell of the medusae). Medusae swam longshore at average speeds ( $5.3 \pm 3.5 \text{ cm s}^{-1}$  SD) that exceeded the local average current speeds ( $2.7 \pm 2.4 \text{ cm s}^{-1}$ ). These and other ecological data were used to parameterise the biophysical model. No medusae modelled as passive were advected from the bay in 14 d; <2.5% of swimming medusae were lost. When medusae swam directionally, a high percentage aggregated in shallow waters within 10s to 100s of m of the seeding locations. Newly metamorphosed medusae are likely to be retained in the bay through a combination of 'sticky water' (i.e. water with reduced current speeds, reduced through diversion around obstacles) in shallow complex habitats and favourable currents. *C. fleckeri* are vulnerable to low salinities; however, modelling a strong flood revealed higher salinity refugia in shallow water. As there was high retention within the system, we conclude that populations of *C. fleckeri* inhabiting shallow, semi-enclosed estuarine bays probably represent stocks. Within these stocks, swimming and favourable currents may minimise connectivity and maintain populations at multiple spatial scales.

**KEY WORDS:** Cubozoa · Population dynamics · Connectivity · Biophysical model · Behaviour

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## INTRODUCTION

Most cubozoan jellyfishes inhabit coastal or reefal waters in the tropics, putting them in close proximity to humans. This is particularly true for chirodropids such as *Chironex fleckeri*, which is only found close to shore in a few metres of water (Gordon & Seymour 2009, Kingsford & Mooney 2014). *C. fleckeri* and the Irukandji jellyfishes have extremely potent venom, and stings can be fatal (Fenner 2005). Additionally, publicity following stinging events can cause a drop in tourism revenue (Gershwin et al. 2010). Despite

the threat they pose to humans, cubozoans are an understudied taxa, and we know little about their population ecology (Kingsford & Mooney 2014).

Cubozoans have a bipartite life history, with a benthic polyp phase and a pelagic medusa phase. *C. fleckeri* polyps are thought to reside in estuaries during the dry season and metamorphose into medusae with the onset of the wet season (Hartwick 1991). Polyps may also inhabit coastal waters (Mooney & Kingsford 2012). The presence of medusae is highly seasonal (Gordon & Seymour 2012, Kingsford & Mooney 2014), with metamorphosis from polyps oc-

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curing throughout the medusae season (Gordon & Seymour 2012). As medusae are pelagic and highly mobile, they have a greater potential for dispersal than the earlier life history stages (from the zygotes to the recently metamorphosed juvenile medusae; Hartwick 1991).

Scales of connection between cubozoan populations within their geographic ranges are unknown. A metapopulation often corresponds with the biogeographic range of the species (Sinclair 1988, Kingsford & Battershill 1998) and is made up of a collection of mesopopulations (often called 'stocks' in a fisheries context; Kingsford et al. 2000). In turn, stocks can be made up of connected 'local populations' (Kingsford & Battershill 1998). It is assumed that robust stocks have limited exchange among them, and that the size of these stocks would largely depend on intrinsic factors such as reproductive output, recruitment, growth and mortality (Sinclair 1988).

For *C. fleckeri*, there may be one or more metapopulations in the species' biogeographic range, which includes Southeast Asia and Northern Australia (Bentlage et al. 2009). The identity of stocks is yet to be determined, but *C. fleckeri* medusae inhabit spatially complex regions, including winding coastlines, nearshore islands, estuaries and embayments. In these environments, oceanographic processes such as trapping in shallow waters and the limited flushing of bays have the potential to keep populations of medusae separate from each other (e.g. Pitt & Kingsford 2000, Mooney & Kingsford 2016a, 2017). Such processes, in combination with the high mobility of medusae (Gordon & Seymour 2009), may limit immigration and emigration between populations. Mooney & Kingsford (2016a, 2017) recently presented evidence that the elemental signatures and shapes of statoliths in *C. fleckeri* medusae varied at spatial scales of 10s to 100s of km. They concluded that local populations, and even stocks, may be divided at surprisingly small spatial scales. An understanding of behavioural ecology and local oceanography would help to explain these patterns.

Other approaches have been used to determine levels of connectivity among population units of jellyfishes, including demographics (e.g. Pitt & Kingsford 2000), genetics (e.g. Dawson 2005, Glynn et al. 2015, van Walraven et al. 2016) and biophysical modelling (e.g. Johnson et al. 2005, Barz et al. 2006, Chen et al. 2014, Wei et al. 2015). Each of these techniques, individually or in combination, can assist in estimating connectivity in ecological and evolutionary time. Biophysical models couple hydrodynamic and behavioural models. They are increasingly being used to

examine the connections between populations (e.g. Quinlan et al. 1999, Paris et al. 2007). Biophysical models of jellyfish have largely included passive medusae (e.g. Moon et al. 2010) or medusae with only simple vertical migration behaviours (e.g. Berlin et al. 2013, Wei et al. 2015, Wu & Xu 2016). Medusae have rarely been programmed with horizontal swimming behaviour. Notably, Fossette et al. (2015) programmed *Rhizostoma octopus* with horizontal swimming behaviour and demonstrated that this behaviour was integral to the maintenance of blooms.

The inclusion of behaviour is critical if the organisms being modelled have the capacity to influence their dispersion (Simpson et al. 2013, Wolanski & Kingsford 2014, Fossette et al. 2015); this is certainly the case for *C. fleckeri* medusae as they are highly mobile and have sophisticated sensory systems. *C. fleckeri* are the largest cubozoans in the world (Kingsford & Mooney 2014). They have sensory receptors including statocysts for balance and complex eyes that enable orientation (Coates & Theobald 2003, Nilsson et al. 2005). Hamner et al. (1995) demonstrated avoidance behaviour in *C. fleckeri*, where medusae swam away from black objects placed at the ends of tanks and manoeuvred around black pipes distributed throughout tanks. Medusae ranging in size from 4.5 to 10 cm bell diameter have been recorded swimming at velocities ranging from 3.6 to 11.5 cm s<sup>-1</sup> in a laboratory setting (Shorten et al. 2005). Colin et al. (2013) analysed video of 4 *C. fleckeri* medusae, with interpedalial distances (the distance between two adjacent corners on the bell of the medusae) of 2 to 16 cm; the medusae swam at maximum speeds of 5 to 12 cm s<sup>-1</sup>. Furthermore, medusae ranging in size from 9 to 17.5 cm interpedalial distance have been recorded travelling 100s of m to km within a day in the wild, and there is some evidence from tagged individuals that they stay within 10s to 100s of m from the shore (Gordon & Seymour 2009).

There is, however, an inherent risk associated with living in shallow coastal waters; namely, rapid decreases in salinity from local runoff. The sensitivity of *C. fleckeri* to low salinities has been demonstrated in several studies. Hartwick (1991) documented the collapse of a population of *C. fleckeri* polyps which coincided with the arrival of freshwater runoff following heavy rain. Kingsford et al. (2012) sampled cubozoan medusae over 3 seasons and found *C. fleckeri* medusae were rare or absent when riverine discharge was high. Mooney & Kingsford (2016b) demonstrated experimentally that *C.*

*fleckeri* medusae were incapacitated at salinities between 21 and 16 practical salinity units (PSU), and the medusae mortality rate was >50% at salinities <16 PSU. Further, Llewellyn et al. (2016) attracted medusae to lights and recorded their occurrence over 5 yr. In the summer months, *C. fleckeri* were never recorded at salinities below 25.2 PSU, although monsoonal rains periodically reduced the salinity to as low as 17.7 PSU. Freshwater pulses, therefore, have the potential to affect the size and persistence of *C. fleckeri* populations.

The objective of this study was to apply a biophysical model to determine if *C. fleckeri* medusae can maintain localised populations at medium (10s of km) to small (100s of m) spatial scales. The specific aims were to (1) quantify the swimming speed and behaviour of medusae in relation to currents, obstacles and the shoreline; (2) generate a biophysical model to determine the importance of medusae mobility in maintaining localised populations; (3) sequentially vary the parameters in the biological component of the biophysical model to verify the reliability of the modelling results; (4) determine the threat that pulses of fresh water from storm events can pose to populations of medusae, and identify strategies medusae could use to survive such events.

## MATERIALS AND METHODS

### Study site

The study site was Port Musgrave, Cape York Peninsula. Port Musgrave is a ~17 × 21 km shallow bay, with a maximum depth of ~12 m (Fig. 1c). The bay is semi-enclosed, with a narrow ~3.5 km mouth which, along with many other bays, opens into the Gulf of Carpentaria in the tropical north of Australia. Behavioural studies of *Chironex fleckeri* were done at Red Beach Mapoon within Port Musgrave (12° 01' 7.43" S, 141° 54' 17.78" E). The salinity at Red Beach was measured on 16 December 2015 using a conductivity, temperature and depth device (CTD; Seabird SBE 19 Plus). We determined the level of stratification in Port Musgrave. Vertical temperature and salinity profiles were measured on 2 occasions at Red Beach and along a transect from Red Beach, across the middle of the bay and to the other side on 15 and 16 December 2016 using a conductivity data logger (HOBO U24-002-C).

### Swimming velocity and behaviour of jellyfish

The swimming speed and behaviour of *C. fleckeri* was determined for medusae ranging in size from 4

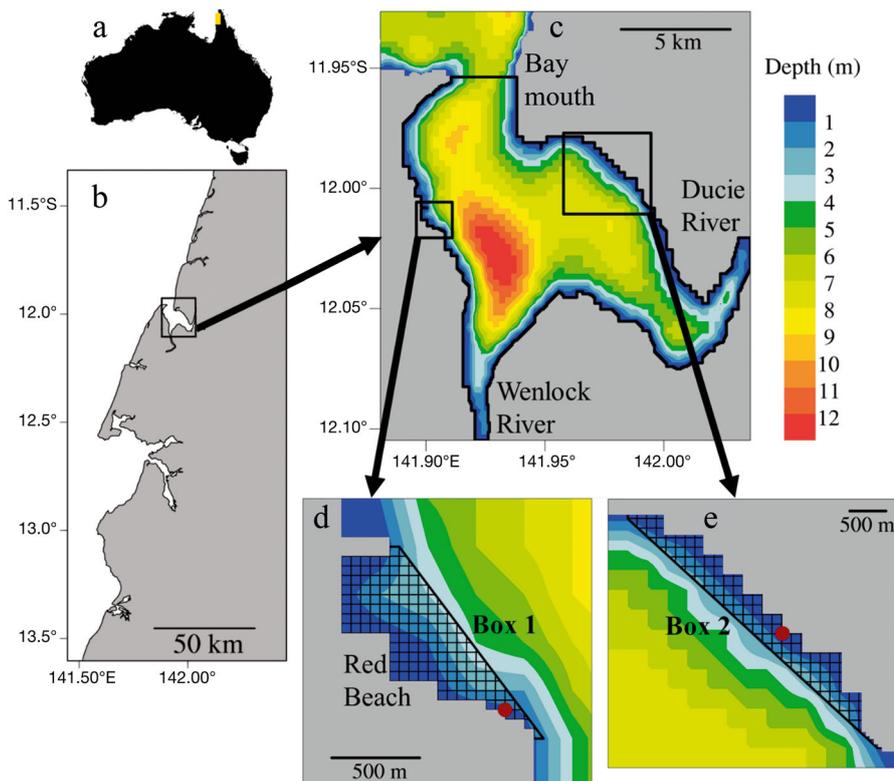


Fig. 1. Study site. (a) Australia; (b) detailed view of the west coast of Cape York Peninsula, which contains multiple estuarine systems including Port Musgrave; (c) bathymetry of Port Musgrave. The number of *Chironex fleckeri* remaining nearshore in the biophysical modelling scenarios were counted in (d) Box 1 on the west side of the bay (adjacent to Red Beach) and (e) Box 2 on the east side of the bay. Medusae were seeded from the locations marked by the red circles

to 12 cm interpedalial distance. Observations on a total of 22 jellyfish were made from 12 to 14 December 2015 at a sandy beach near mangrove habitat when the tide was high; medusae were in shallow water <0.5 m deep. Swimming speeds were determined by placing the weighted end of a transect tape near each medusa without disturbing it. An observer then walked parallel and slightly behind the medusa, with a separation distance of approximately 3 m. None of the medusae overtly reacted to the presence of the observer. The tape was used to measure the distance travelled in 30 s intervals for 2 to 10 min. Medusae generally swam parallel to the edge of the beach. Trials were discontinued after a minimum of 2 min when jellyfish disappeared due to poor visibility or, in one case, when a medusa veered seaward into water over 1 m deep. The mean current velocity was then determined by measuring the distance a natural float (e.g. submerged leaf or seagrass fragment) travelled through time ( $n = 2$  medusa<sup>-1</sup>). The swimming orientation of the medusa with respect to the current and the beach was noted. The swimming velocity over ground was determined from the swim speed and orientation. The velocity through water was calculated by subtracting the current velocity from the velocity over ground when the medusae swam with the current, and calculated by adding the current velocity if they swam against it.

The avoidance behaviour of *C. fleckeri* medusae was investigated experimentally using medusae ranging in size from 3 to 11 cm interpedalial distance. *C. fleckeri* were observed from 14 to 16 December 2016. There were 2 treatments in the design: (1) with obstruction and (2) a control without an obstruction. For Treatment 1, the paths of 7 medusae were obstructed by an observer who stood 2 to 6 m in front of them. The observer estimated the distance at which the medusa performed an avoidance manoeuvre and the angle of deviation. For Treatment 2, 7 unobstructed medusae were observed to determine natural deviations in swimming direction. These medusae were observed for up to 6 min and the distance they travelled in a straight course was estimated. A course was considered straight if it deviated  $\leq 20^\circ$  from the initial heading.

### Biophysical modelling

The biophysical model of Port Musgrave consisted of a hydrodynamic model coupled with a dispersion model that incorporated medusae swimming behaviour. The hydrodynamic model of Wolanski & Kings-

ford (2014) was used to simulate the circulation in Port Musgrave. It is a 2-dimensional (depth-averaged) finite-difference model which solves the Navier-Stokes equations (Black et al. 1991). Two-dimensional depth-averaged models realistically recreate observed currents in shallow water (Lambrechts et al. 2008). The waters of Port Musgrave are shallow and vertically well mixed in salinity and temperature during the dry season (Wolanski 1986) and at the beginning of the wet season (see 'Results'), justifying the use of a 2-dimensional model to simulate the currents. The model was forced with tides, wind and in some scenarios, river discharge. The mouth of the bay was the seaward open boundary. Port Musgrave faces the Gulf of Carpentaria where the longshore currents in coastal waters are weak (Wolanski 1993); further, the mouth of Port Musgrave is narrow. We assumed, therefore, that the longshore currents in the gulf have little influence in Port Musgrave.

Port Musgrave has mixed semi-diurnal tides that alternated between spring (12 December 2015), average (waxing crescent moon; 17 December 2015), and neap (19 December 2015) phases. These 3 tidal scenarios were reconstructed from the predictions of the software AusTide 2015 v.1.10.1 and forced at the seaward open boundary.

In northern Australia, the *C. fleckeri* season usually runs from October to April ([www.aims.gov.au/docs/projectnet/sea-wasp.html](http://www.aims.gov.au/docs/projectnet/sea-wasp.html)). The average ( $\pm$ SD) wind measured at Weipa Airport (12° 40' 40.08" S, 141° 55' 14.88" E, ~70 km from Port Musgrave) over 10 jellyfish seasons (from 2005–2006 to 2014–2015) was  $2.63 \pm 1.92$  m s<sup>-1</sup> and the average wind direction was from the east (89.14°). These average conditions were imposed as a constant wind to force all model scenarios. We ran models with and without a wind shadow area on the leeward side of the bay (east). As wind shadow had little effect on the dispersion of medusae, wind shadow was not included in the scenarios presented in the study. The wind data were provided by the Australian Government Bureau of Meteorology.

Port Musgrave is in tropical north Queensland; the region gets little rain in the dry season (May to November) and monsoonal rain in the wet season (December to April). The Wenlock and Ducie Rivers discharge into the bay. Flow rate data for the Wenlock were recorded at Jacks Camp (12° 24' 32.5" S, 142° 18' 16.9" E, ~102 km from the river mouth) between March 1971 and May 1988. Flow rate data for the Ducie were recorded at Bertiehaugh (12° 07' 37.4" S, 142° 22' 31.6" E, ~55 km from the river mouth) between December 1968 and September

1988. These data were sourced from the Queensland Government water monitoring information portal (<https://water-monitoring.information.qld.gov.au/>). A discharge of  $1000 \text{ m}^3 \text{ s}^{-1}$  from both rivers for 5 d was identified as a realistic wet season flood event (see Figs. S1 & S2 in the Supplement at [www.int-res.com/articles/suppl/m12305\\_supp.pdf](http://www.int-res.com/articles/suppl/m12305_supp.pdf)), and was simulated in the flood scenarios.

Bathymetry data were derived from the Geoscience Australia, Australian Bathymetry and Topography 2009 data set which has a resolution of  $\sim 250 \text{ m}$ . A bilinear interpolation was performed on the bathymetry data to increase the resolution to  $55 \text{ m}$  for use in the model. The hydrodynamic model had a time step of  $2 \text{ s}$  and the output was saved every  $30 \text{ min}$  and used to run the advection–dispersion model.

Medusae were seeded as particles in the advection–dispersion model. It was assumed that the medusae exported seaward out of Port Musgrave were unable to return to the bay. It was also assumed that the medusae that advected onto dry cells beached and died, though this is very conservative as the behaviour of medusae suggests that beaching is unlikely. Medusae were either set to be passive (Behaviour A) or to

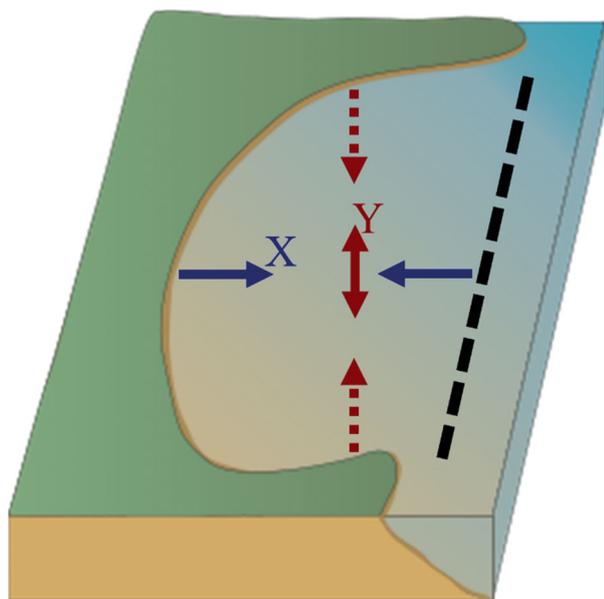


Fig. 2. Schematic of the *Chironex fleckeri* medusae behaviours included in the biophysical model. Red arrows: behaviours in the Y direction; blue arrows: behaviours in the X direction. Solid arrows: swimming behaviours included in Behaviour B (swimming back and forth, parallel to shore, in the Y direction, and swimming to shore and avoiding beaching in the X direction); Dashed arrows: additional behaviour included in Behaviour C (avoidance behaviour, with a directional reversal of  $180^\circ$  in the Y direction). Behaviours were only carried out if medusae were within the behavioural band, as denoted by the blank dashed line

swim according to assigned behaviours (Behaviours B and C; Fig. 2). The behaviours were assigned using scalar fields. Separate fields were generated for behaviour in the X (perpendicular to the shore) and Y (parallel to the shore) directions. The value of each cell in the field designated the direction medusae would swim when in that cell. For Behaviour B, medusae were made to swim back and forth in the Y direction, as they were observed doing at Red Beach. This was achieved by generating random numbers between 0 and 1 at each cell in the scalar field. If the number was  $< 0.5$  the medusae were made to swim south, otherwise they swam north. In the X direction, medusae swam back toward the shore. This behaviour was included because a combination of prevailing currents and behaviour can disperse medusae 100s of m to km within a day (Gordon & Seymour 2009). If medusae did not swim directionally toward the shore, they would be dispersed seaward into deeper waters where stronger currents persist (see Fig. S3 in the Supplement), contradicting their observed distribution in predominantly shallow waters, less than a few hundred metres from the shore (Gordon & Seymour 2009, Kingsford & Mooney 2014). In the first line of wet cells, which surround the coastline, medusae were set to swim away from the shore to avoid beaching. A new condition was added to Behaviour B to generate Behaviour C. Medusae were seeded in small bays that had a U shape. For Behaviour C, when they reached the ends of these bays and encountered mangroves they were made to perform a  $180^\circ$  turn and swim back into the bays. This behaviour represents the avoidance response we document in this paper, and our findings concur with the observations of Hamner et al. (1995) of *C. fleckeri* avoiding obstacles, with directional reversals of up to  $180^\circ$ . The responses we documented are also consistent with the considerable avoidance and orientation capabilities documented in other cubozoan species (Garm et al. 2007, 2011). Behaviours B and C were only carried out within the first 5 cells from shore, which corresponded to  $\sim 275 \text{ m}$ . This is conservative given that Gordon & Seymour (2009) observed a medusa staying within  $300 \text{ m}$  of the shore over a 26 h period (Fig. S3 in the Supplement).

The movement of medusae was calculated as the sum of 3 vectors: the water velocity, the swimming velocity and a random diffusion vector representing horizontal turbulent mixing at sub-grid scales (Paris et al. 2002). The average ( $\pm \text{SD}$ ) swimming velocity through water calculated for the *C. fleckeri* observed in this study ( $5.3 \pm 3.5 \text{ cm s}^{-1}$ ) was used in the model, as it best represents the swimming capabilities of medusae in the size range sampled. The behaviour of

Table 1. Modelled scenarios and results. The tidal forcing, the programmed behaviour of the *Chironex fleckeri* medusae (see 'Materials and methods: Biophysical modelling' for descriptions of Behaviours A, B and C), the simulated discharge from the Wenlock and Ducie rivers, the diffusion coefficient ( $k_x$ ), the duration of the model run and the percentage of modelled medusae remaining in Boxes 1 and 2 (see Fig. 1d,e), and in Port Musgrave are shown

Scenario no.	Tide	Behaviour	Discharge ( $\text{m}^3 \text{s}^{-1}$ )	$k_x$	Days	% Box 1	% Box 2	% Bay
<b>No flood</b>								
1	Spring	A	0	0.02	14	2.3	0	100
2	Avg	A	0	0.02	14	1.1	0	100
3	Neap	A	0	0.02	14	3.2	0	100
4	Spring	B	0	0.17	14	65.4	0.6	94.8
5	Avg	B	0	0.17	14	73.2	0.5	97.6
6	Neap	B	0	0.17	14	68.3	0.6	99.0
7	Spring	C	0	0.17	14	60.8	89.0	95.7
8	Avg	C	0	0.17	14	68.3	81.6	98.4
9	Neap	C	0	0.17	14	63.4	84.7	99.6
<b>Flood</b>								
10	Avg	A	1000	1	5	1.1	7.1	79.2
11	Avg	C	1000	1	5	14.8	56.3	79.8

medusae determined the swimming direction. The random diffusion vector was calculated as a Markov diffusion process, parameterised by the horizontal diffusion coefficient  $k_x$ , following Spagnol et al. (2002). The exact value of  $k_x$  is unknown. For a grid size of 55 m,  $k_x \approx 0.02 - 0.17 \text{ m}^2 \text{ s}^{-1}$  (Okubo 1971, Wolanski 1992). During storm events that generate floods, there is greater capacity for diffusion given the increased turbulence in the system (Wolanski & Elliott 2015). Consequently,  $k_x$  was increased to  $1 \text{ m}^2 \text{ s}^{-1}$  in the flood scenarios.

In scenarios 1 to 9 (listed in Table 1), all combinations of tide and behaviour were modelled to determine how patterns of dispersion vary by condition. Medusae were seeded from Red Beach, where the biological data were collected, and from another site on the opposite side of the bay (Fig. 1d,e). A total of 25 000 virtual medusae were seeded per site. The scenarios were run for 14 d to determine the degree to which jellyfish were retained within the system. No river discharge was simulated in these scenarios, which is realistic given the flow rates of the Wenlock and Ducie Rivers are often  $<10 \text{ m}^3 \text{ s}^{-1}$  for extended periods of time during the medusae season (Figs. S1 & S2 in the Supplement).

#### Relative importance of swim speed and the width of the behaviour band

The robustness of our outcomes from the model were tested with a sensitivity analysis (SA). A series

of model scenarios (SA1 to SA9; Table 2) were run, each having the same hydrodynamic forcings of average tide and wind, and no river outflow. Run SA5 was the standard run, in which Behaviour C medusae were set to swim at the average speed measured in the field, and could implement a behavioural response within  $\sim 275 \text{ m}$  from the shore, as in scenario 8 (Tables 1 & 2). The behavioural parameters in the other runs were changed as follows. One standard deviation was either added to or removed from the swim speed, making it  $1.8$  or  $8.8 \text{ cm s}^{-1}$  respectively. The width of the behaviour band was either 'halved' to 3 cells ( $\sim 165 \text{ m}$ ) or doubled to 10 cells ( $\sim 550 \text{ m}$ ). All combinations of swim speed and behaviour band width were modelled. The sensitivity analysis runs are listed in Table 2 and they address specific aim 3 (see final paragraph of 'Introduction'). They were analysed separately from the model scenarios listed in Table 1, which address the specific aims 2 and 4.

#### Fresh water pulse flood events

It was hypothesised that pulses of freshwater pose a threat to medusae through increasing their seaward export and decreasing the salinity. In scenarios 10 and 11, passive and Behaviour C medusae were seeded in flood conditions. These scenarios were run to determine how the changed hydrodynamic environment would affect the retention potential of me-

Table 2. Sensitivity analysis (SA) scenarios and results. The width of the behaviour band (BB), swimming speed of the *Chironex fleckeri* medusae, and the percentage of modelled medusae remaining in Boxes 1 and 2 (see Fig. 1d,e), and in Port Musgrave are shown

Scenario no.	BB width (m)	Speed ( $\text{cm s}^{-1}$ )	% Box 1	% Box 2	% Bay
SA1	165	1.8	4.3	0.9	99.5
SA2	165	5.3	3.2	1.3	99.0
SA3	165	8.8	7.1	3.6	99.6
SA4	275	1.8	24.3	10.7	97.1
SA5/Scenario 8	275	5.3	68.3	81.6	98.4
SA6	275	8.8	32.6	93.6	99.7
SA7	550	1.8	67.5	31.1	99.5
SA8	550	5.3	96.7	99.9	100
SA9	550	8.8	93.3	100	99.9

dusae. The effect of the flood on the salinity of the bay waters was then calculated to determine if *C. fleckeri* medusae remaining in the bay could survive such an event, given their sensitivity to low salinities (Mooney & Kingsford 2016b). The average tide and wind were used to force the hydrodynamic model. Again, 25 000 virtual medusae were seeded from both the east and west sides of Port Musgrave. The medusae were set to swim at average speed within a band adjacent to the shore, ~275 m wide. Freshwater discharges of  $1000 \text{ m}^3 \text{ s}^{-1}$  were simulated from both the Wenlock and Ducie Rivers for 5 d, as described previously. It was assumed that the middle of each river plume contained fresh water, with a salinity of 0 PSU, as demonstrated in the flood plumes of tropical estuaries following heavy river discharge (Chevalier et al. 2014). The mean ( $\pm$ SD) salinity at Red Beach was  $35.57 \pm <0.01$  PSU ( $n = 2$ ) when the watershed had received little rain in the week preceding the measurements; it was assumed that cells containing no virtual water particles had this salinity. To calculate the salinity of the virtual flood plume, the salinity was assumed to increase linearly with decreasing particle concentration, from the particle concentration corresponding to 0 PSU to the absence of particles which was estimated to be 35.57 PSU.

### Data analysis

It was possible that the swimming speed of jellyfish would vary with size. Accordingly, the relationship between medusa interpedalial distance and average swim velocity through water was tested using a Spearman's rank correlation, because the data did not meet the assumption of the Pearson's correlation of a bivariate normal distribution.

Retention on a medium spatial scale (i.e. whole bay) was compared between scenarios by counting the number of medusae remaining in Port Musgrave at the end of each of the model runs. Furthermore, retention on a small spatial scale was compared between scenarios by tracking the number of virtual jellyfish remaining in the small bays where they were seeded. The number of medusae remaining in water generally <3 m deep were counted in Box 1 (~0.5 km<sup>2</sup>; Fig. 1d) on the west side of the bay, and Box 2 (~1.9 km<sup>2</sup>; Fig. 1e) on the east side of the bay.

## RESULTS

### Swim velocity and behaviour

*Chironex fleckeri* medusae swam at an average ( $\pm$ SD) velocity through water of  $5.3 \pm 3.5 \text{ cm s}^{-1}$  while being tracked (167 time intervals for 22 jellyfish). The swimming performance of the jellyfish was about 2 times faster than the average current velocity near the beach of  $2.7 \pm 2.4 \text{ cm s}^{-1}$  ( $n = 40$ ). Current speeds were low, but varied from 0 to  $10 \text{ cm s}^{-1}$ . Current speeds along the beach varied according to an interaction between wavelets (<5 cm high) and minor deviations in the beach shape. Faster currents were 'mini rips' that generally only affected a few metres of beach.

There was a poor but significant negative correlation between medusa interpedalial distance and their average swim velocity through water (Spearman's rank correlation,  $r_s = -0.44$ ,  $z_{(2)} = -2.03$ ,  $p < 0.05$ ,  $n = 22$ ). Medusae ranging in size from 4 to 8.5 cm interpedalial distance had an average swim velocity through water of  $7.0 \pm 3.2 \text{ cm s}^{-1}$  (Fig. 3b). Larger medusae, ranging in size from 9 to 12 cm interpedalial distance, had a slower average swim velocity through water of  $3.9 \pm 2.1 \text{ cm s}^{-1}$ . The smallest individual observed in this study had an interpedalial distance of 4 cm and swam at a maximum speed of  $6.5 \text{ cm s}^{-1}$ . The fastest speed for an individual within a 30 s interval was  $16.6 \text{ cm s}^{-1}$ , recorded for a medusa with an interpedalial distance of 6 cm.

Of the 22 observed medusae, 21 initially tracked along the beach, 40.91% were swimming with the longshore current and 54.55% were swimming

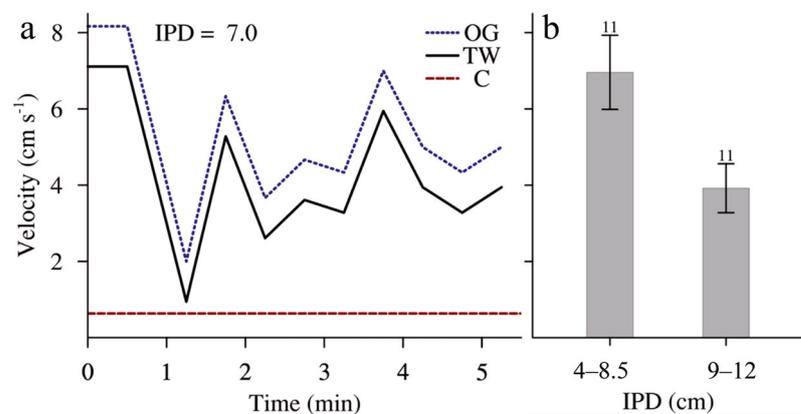


Fig. 3. (a) Velocity of an individual *Chironex fleckeri* medusa through time, showing the current velocity (C), the velocity over ground (OG) and the velocity through the water (TW). (b) Average ( $\pm$  SE) velocity through water (of medusae grouped by interpedalial distance, IPD). The number of medusae in each size class are shown above the error bars

against it. Only 1 medusa (4.55%) was initially swimming perpendicular to the longshore current, away from shore.

Medusae performed avoidance manoeuvres when they encountered an obstruction (the observer). The average distance of response was  $1.4 \pm 0.4$  m from the observer. Medusae altered their heading by an average of  $83 \pm 47^\circ$  (range 45 to  $180^\circ$ ). In the control treatment without an obstacle, medusae maintained a straight course parallel to the beach for an average distance of  $8.1 \pm 5.6$  m.

### Hydrodynamics of Port Musgrave

The hydrodynamic model revealed significant current shear, where the currents increased with distance from shore. For example, from Red Beach at peak ebb during the average tide, the currents increased along a transect from  $0.5 \text{ cm s}^{-1}$  55 m from shore to  $7.8 \text{ cm s}^{-1}$  550 m from shore (Fig. 4). The waters of Port Musgrave were not stratified at the beginning of the wet season. In waters shallower than 5 m, variation in temperature of the water column was  $<0.53^\circ\text{C}$  and salinity was  $<0.31$  PSU. In waters  $>5$  m and to a depth of 10.8 m, the temperature varied by  $<0.27^\circ\text{C}$  and the salinity varied by  $<0.45$  PSU. In dry conditions, the tidally averaged net circulation consisted of an inflow over the shallows and an outflow in the deeper waters around the axis of the bay. The inflow took  $\sim 10$  to 14 d to reach the headwaters. This same amount of water exits the bay

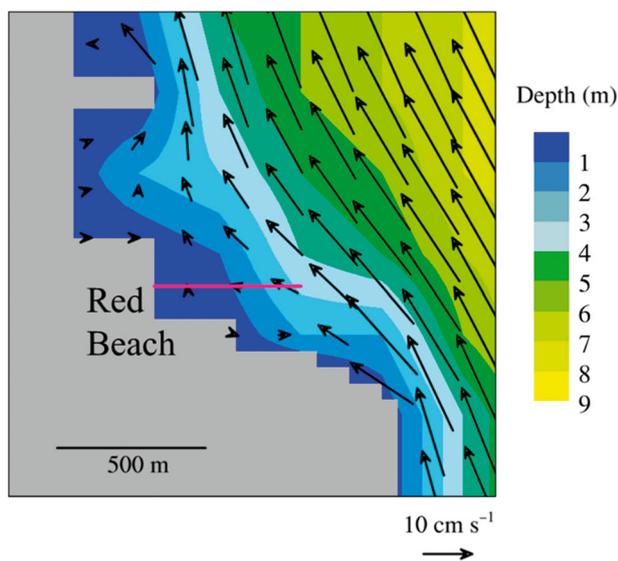


Fig. 4. Modelled velocity field at Red Beach (see Fig. 1d) at peak ebb tide. Velocities were reported from the ends of the transect shown with the pink line

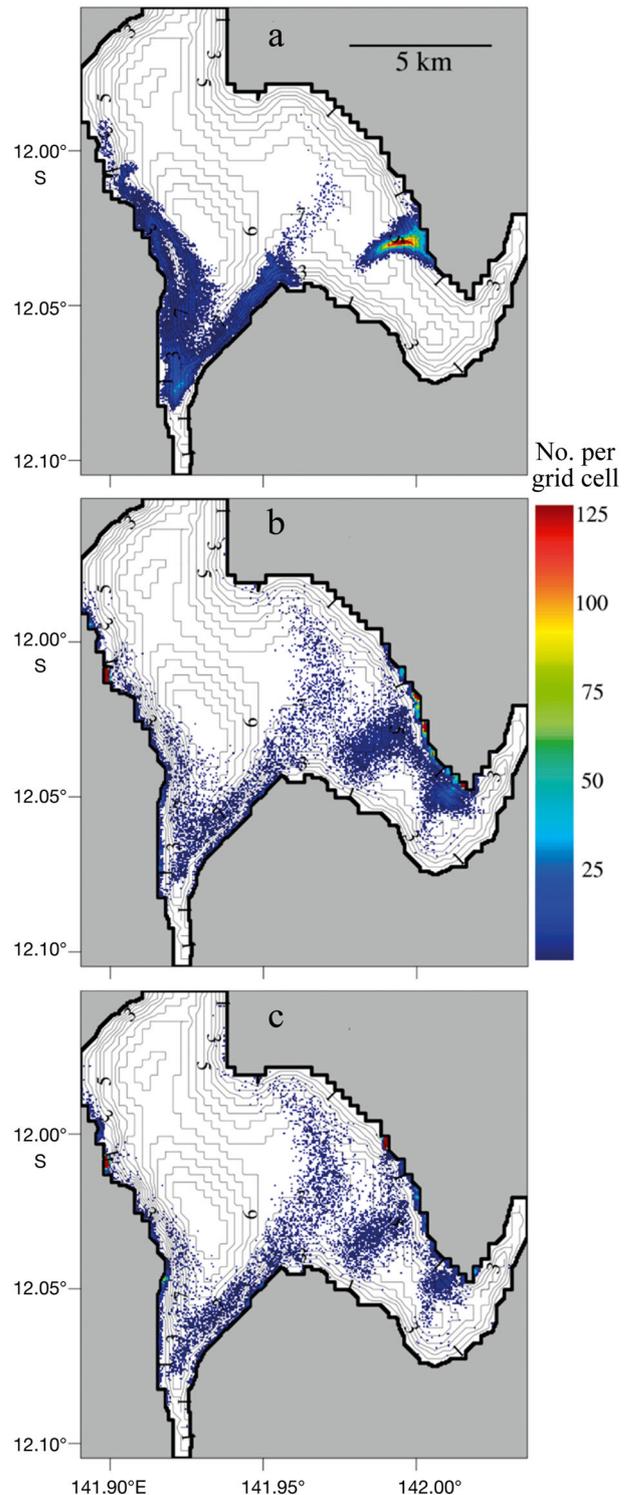


Fig. 5. Number of *Chironex fleckeri* medusae per grid cell with (a) Behaviour A (passive; scenario 2), (b) Behaviour B (scenario 5) and (c) Behaviour C (scenario 8) at the end of the model runs. In these scenarios, runs lasted for 14 d after the particles had been released from the seed locations (see Fig. 1d,e). The model was forced with average tides. No freshwater outflow from the rivers were included (i.e. 'no flood'; Table 1). Depth contours are indicated

in the deeper parts, which are typically 3 times the depth of the shallows. Because mass is conserved the outflow would take 3 times longer, ~30 to 42 d. Thus, in dry conditions, the residence time of Port Musgrave is ~40 to 56 d.

### Retention – dry conditions

In all behavioural scenarios, the net current transported some medusae from the west side to the east side of Port Musgrave (Fig. 5). At both medium (km) and small scales (i.e. individual beaches, 10s to 100s of m) the tidal conditions made little difference to the level of retention. The numbers of virtual medusae

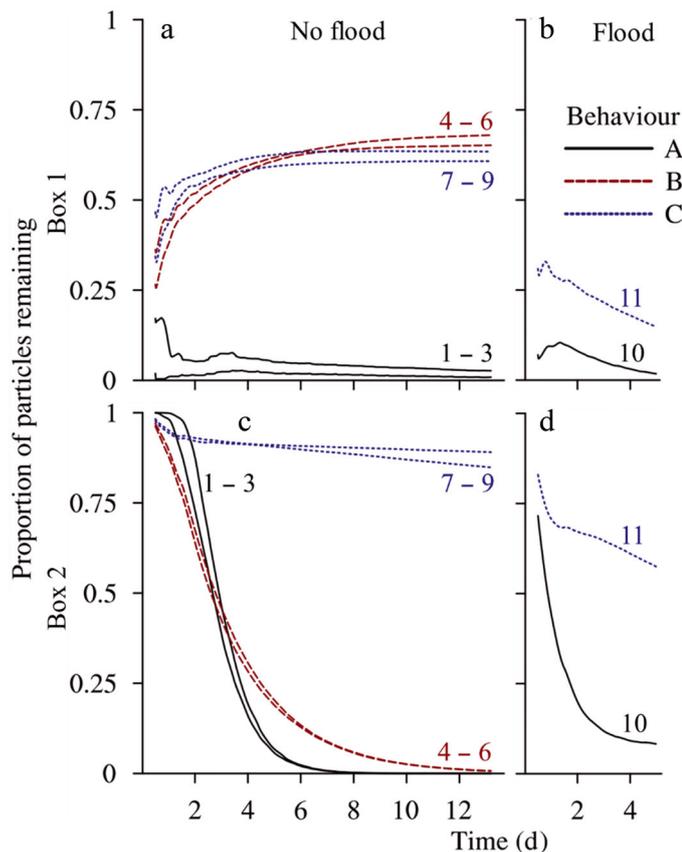


Fig. 6. Proportion of virtual *Chironex fleckeri* medusae remaining near shore through time for the modelling scenarios listed in Table 1. Scenario numbers are shown. (a) Retention in Box 1 (see Fig. 1d), no flood scenarios; (b) Box 1, flood scenarios; (c) retention in Box 2 (see Fig. 1e), no flood scenarios; (d) Box 2, flood scenarios. For the no flood scenarios, the 2 lines for each behavioural category show the range of proportions obtained among the different tidal forcings. Active medusae (Behaviours B and C) swam at  $5.3 \text{ cm s}^{-1}$  (average) and performed the prescribed behaviours within a 275 m band from shore. A 24 h running average was performed on the raw data to remove tidal variability

remaining in Port Musgrave after 14 d were similar among the different tidal scenarios and similar numbers were retained in Boxes 1 and 2 through time (Fig. 6a,c, Table 1).

At a spatial scale of 10s of km, no virtual medusae were lost from Port Musgrave after 14 d when they were treated as passive particles in dry conditions (no flood, Behaviour A; scenarios 1 to 3; Table 1). Very low loss rates were found when behaviour was included:  $\leq 5.2\%$  of the Behaviour B medusae were lost (scenarios 4 to 6) and  $\leq 4.3\%$  of Behaviour C medusae were lost (scenarios 7 to 9).

At a smaller spatial scale (100s of m), passive medusae (scenarios 1 to 3) were quickly advected away from the release points on both the west and east sides of the bay (Figs. 5 & 6a,c). After 14 d, few were retained in Box 1 ( $< 3.2\%$ ) and no medusae were in Box 2 (Table 1).

The behaviour of medusae had a great influence on nearshore retention. On the west side of the bay,  $> 50\%$  of Behaviour B medusae (scenarios 4 to 6) were initially advected out of Box 1, but they swam back into the small bay over the course of a few days. At the end of 14 d, between 65.4 and 73.2% remained. In contrast, Behaviour B medusae were quickly advected from Box 2 on the east side of the bay with only between 0.5 and 0.6% remaining after 14 d. The net current advected the medusae plume south and medusae were subsequently retained in high numbers at some sheltered locations on the jagged eastern coastline,  $< 5 \text{ km}$  from Box 2. The addition of avoidance behaviour slightly decreased retention in Box 1. Again, over 50% of medusae (Behaviour C, scenarios 7 to 9) were initially advected out of the Box but they quickly swam back in and between 60.8 and 68.3% remained after 14 d. The retention in Box 2 greatly improved when avoidance behaviour was included, with between 81.6 and 89% remaining at the end of the 14 d run.

### Sensitivity analysis

At the medium spatial scale of the whole bay, retention was  $> 97\%$  in all scenarios (Table 2). At the small spatial scale (100s of m, near shore waters), retention of medusae was high in the standard run (SA5), where medusae were set to swim at the average speed and make behavioural responses within an intermediate distance from shore (Fig. 7c,d, Table 2). In Box 1, on the west side of the bay, 68.3% of medusae remained after 14 d. Retention was even higher in Box 2, on the east side of the bay, where

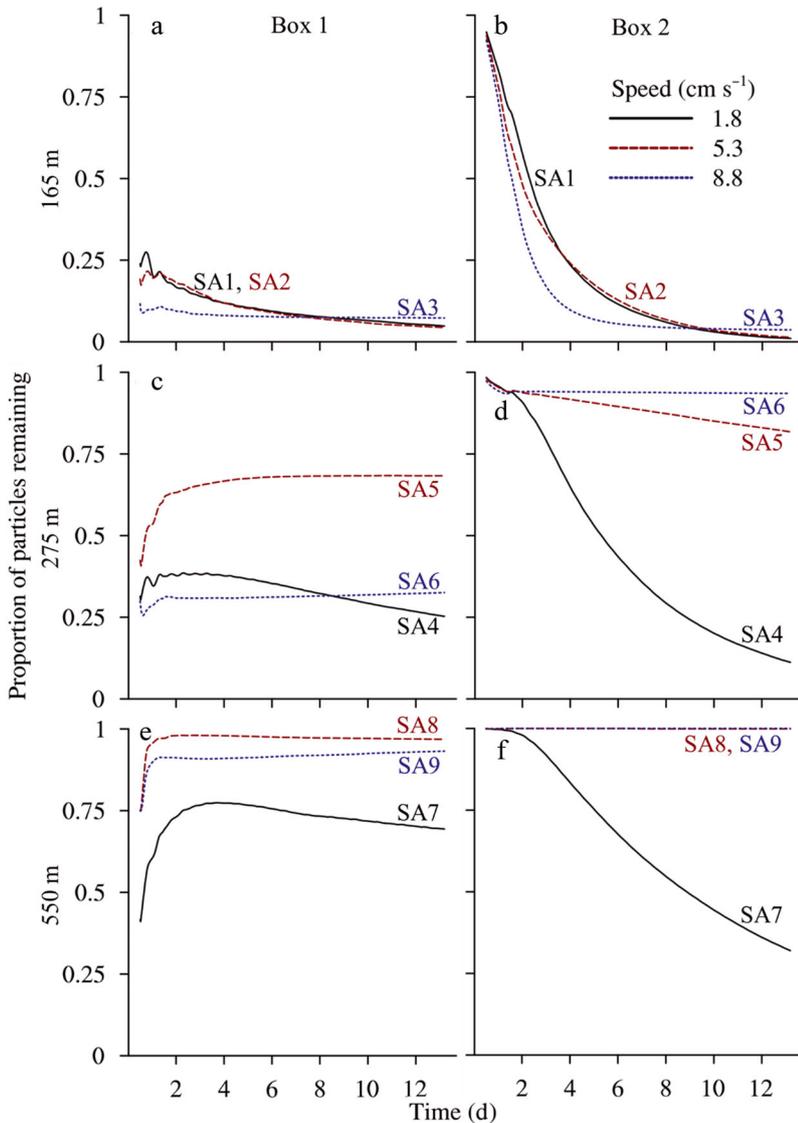


Fig. 7. Proportion of virtual *Chironex fleckeri* medusae remaining near shore through time in the sensitivity analysis (SA) modelling scenarios listed in Table 2. In the first row, the retention in (a) Box 1 (see Fig. 1d) and (b) Box 2 (see Fig. 1e) is shown for scenarios SA1 to SA3 where medusae perform the prescribed behaviours in a 165 m band from shore. In the second row, the retention in (c) Box 1 and (d) Box 2 is shown for scenarios SA4 to SA6 where there is a 275 m band from shore. In the third row, the retention in (e) Box 1 and (f) Box 2 is shown for scenarios SA7 to SA9 where there is a 550 m band from shore. A 24 h running average was performed on the raw data to remove tidal variability

81.6% of medusae remained, although medusae were slowly lost through time.

Reducing the behaviour band width and swim speed below the standard parameterisation was detrimental for retention. The number of medusae remaining in nearshore waters declined through time when the behaviour band width was 165 m, the narrowest setting, irrespective of the swim speed (Fig. 7a,b; scenarios SA1 to SA3). Loss of medusae

was rapid in these scenarios, with  $\leq 7.1\%$  remaining in either Box after 14 d. The number of medusae remaining near shore through time did stabilize when the behaviour band was at its narrowest and they swam at above average speed (SA3), although few medusae were retained (7.1% in Box 1 and 3.6% in Box 2). Numbers declined through time when medusae swam at below average speeds (SA1, SA4 and SA7), irrespective of the behaviour band width. The width of the behaviour band did affect the rate of loss; for example, only 24.3% of slow swimming medusae remained in Box 1 after 14 d when the behaviour band width was 275 m wide and 67.5% remained when it was 550 m wide.

Widening the behaviour band from 275 to 550 m (scenarios SA7 to SA9) improved retention; for example, when the swim speed was average, widening the behaviour band improved retention in Box 1 by 28.4%. Near 100% retention was recorded in Box 2 when medusae swam at average or above average speed and the behaviour band was 550 m wide (SA8, SA9). Increasing the swim speed above the standard parameterisation (SA3, SA6 and SA9) was bad for the retention of medusae in Box 1 and only marginally improved the retention in Box 2. For instance, retention in Box 1 dropped from 68.3 to 32.6% when the behaviour band was 275 m wide and the swim speed was increased from average (SA5) to above average (SA6). In the same scenarios, medusae retention in Box 2 only increased from 81.6 to 93.6% with the increase in swim speed.

### Flood events; retention and refugia

During the strong flood, the salinity in a large proportion of Port Musgrave was reduced to  $< 21$  PSU (Fig. 8). The salinity near the mouth of the bay, in the northernmost region farthest from the rivers, was not reduced from the original bay salinity of 35.57 PSU. Additionally, the salinity in a band of shallow water adjacent to the coastline on each side of the bay

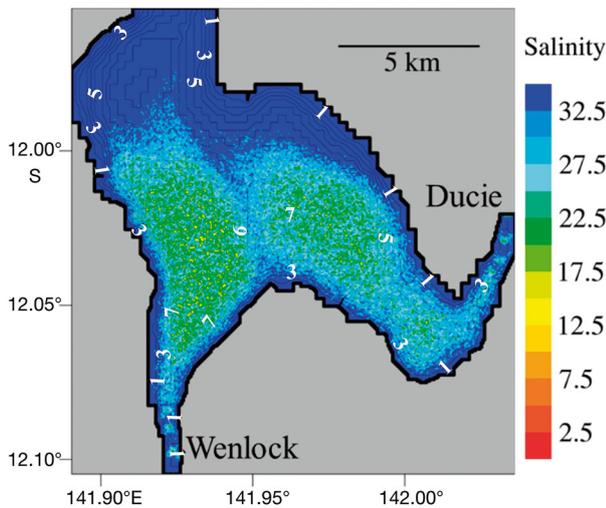


Fig. 8. Salinity within Port Musgrave at peak ebb tide after 5 d of flooding at a rate of  $1000 \text{ m}^3 \text{ s}^{-1}$  from both the Wenlock and Ducie Rivers. Depth contours are indicated

remained above 21 PSU (i.e. above the threshold of risk to *C. fleckeri*) for the duration of the flood event. The additional volume of water and related transport resulted in a 20% reduction in the retention of both passive (dropped from 100 to 79.2%; scenarios 2 and 10, Table 1) and swimming medusae (dropped from

98.4 to 79.8%; scenarios 8 and 11) within Port Musgrave. Passive medusae were quickly advected from nearshore waters on both the east and west sides of the bay (Fig. 6b,d). Only 1.1% of the seeded medusae remained in Box 1 and 7.1% remained in Box 2 after 5 d. When behaviour was included, medusae were still advected from nearshore waters, but after the flood event, 14.8 and 56.3% remained in Boxes 1 and 2 respectively. The average salinity in Box 1 did not fall below 35.41 PSU at any time during the flood event and the average salinity in Box 2 did not fall below 35.57 PSU.

## DISCUSSION

### Swim speed

*Chironex fleckeri* medusae are strong swimmers. The maximum swim speed recorded in this study ( $16.6 \text{ cm s}^{-1}$ ; see 'Results: Swim velocity and behaviour') is among the fastest recorded for any jellyfish (Table 3). It is also greater than the fastest swimming speeds of *C. fleckeri* medusae reported by Shorten et al. (2005) and by Colin et al. (2013), of  $11.5$  and  $12 \text{ cm s}^{-1}$  respectively. Multiple methods have been used to measure the swim speeds of medusae over short time

Table 3. Examples of maximum recorded speeds of jellyfishes (Max. speed). The size of medusae refers to the size reported in the relevant reference and is presented as either interpedalial distance (IPD), bell diameter (BD) or diagonal bell width (DBW). Method: method used to measure speed

Class	Species	Max. speed ( $\text{cm s}^{-1}$ )	Size (cm)	Method	Reference
Cubozoa	<i>Chironex fleckeri</i>	12	16 IPD	Digital video analysis	Colin et al. (2013)
	<i>Chiropsella bronzie</i>	12	5.6 IPD	Digital video analysis	Colin et al. (2013)
	<i>C. fleckeri</i>	11.5	10 BD	Digital video analysis	Shorten et al. (2005)
	<i>Chiropsalmus</i> sp.	6.7	4.5 BD	Digital video analysis	Shorten et al. (2005)
	<i>Carybdea marsupialis</i>	5	3.3 DBW	Digital video analysis	C. Bordehore pers. comm.
	<i>Tripedalia cystophora</i>	4 against 1.5 current	0.8 to 1.2 BD	Flow tank	Garm et al. (2007)
Scyphozoa	<i>Nemopilema nomurai</i>	53	68 BD	Particle tracking velocimetry	Lee et al. (2010)
	<i>Stomolophus meleagris</i>	15	Not reported	Observed in field	Shanks & Graham (1987)
	<i>Phacellophora camtschatica</i>	10.2	25 to 45 BD	Electronic tags	Moriarty et al. (2012)
	<i>Catostylus mosaicus</i>	10	>14 BD	Observed in field	Pitt & Kingsford (2000)
	<i>Linuche unguiculata</i>	8.3	1.4 to 2.2 BD	Digital video analysis	Larson (1992)
	<i>Rhizostoma octopus</i>	8	30 to 40 BD	Electronic tags	Fossette et al. (2015)
	<i>Cyanea capillata</i>	5.7	20 to 35 BD	Electronic tags	Moriarty et al. (2012)
Hydrozoa	<i>Sarsia tubulosa</i>	4.1	0.8 BD	Digital video analysis	Katija et al. (2015)

periods, including digital video analysis (Larson 1992, Shorten et al. 2005, Colin et al. 2013, Katija et al. 2015), flow tank experiments (Garm et al. 2007) and acoustic methods (Lee et al. 2010). In this study, simple distance over time measurements were made in the field over short periods. This technique is particularly useful for *C. fleckeri* medusae due to their extreme nearshore distribution and their tendency to swim parallel to the beach. Pitt & Kingsford (2000) also used this method for estimating the swim speed of *Catostylus mosaicus* in a shallow lake. Using electronic tags to track medusae has the potential to provide great insights into the swimming capabilities of jellyfishes over longer time periods (Fossette et al. 2016). For example, Moriarty et al. (2012) and Fossette et al. (2015) attached electronic tags to medusae and tracked their movements for 24 and 6 h respectively; the swimming speeds of the medusae could be calculated in both studies because the local current speeds had been measured. Electronic tagging of jellyfishes has only recently been made possible due to the difficulties of attaching the tags to soft-bodied invertebrates, and tag retention is often a problem (Fossette et al. 2016).

### Medium scale retention

*C. fleckeri* medusae showed strong swimming behaviour and a preference to stay close to shore. These behaviours, in some cases combined with favourable currents, resulted in a high level of retention at medium (10s of km) and small (10s to 100s of m) spatial scales. It is likely, therefore, that the *C. fleckeri* population inhabiting Port Musgrave has little exchange with other similar estuaries and suitable habitats, and it probably represents a stock. We provided evidence that even medusae behaving as passive particles are unlikely to emigrate out of the system in dry conditions. The geographic configuration, flow regime and high residence time of Port Musgrave must play a large role in controlling the dynamics of the resident *C. fleckeri* population. Port Musgrave is a relatively closed system, with only a narrow connection to the Gulf of Carpentaria, and the flow into the bay from the Wenlock and Ducie Rivers is negligible for a large portion of the *C. fleckeri* medusae season. There are several estuaries and bays of similar size and geomorphology within the Gulf of Carpentaria and at other tropical locations in northern Australia and Oceania, thus our findings may have broad applicability. Like Port Musgrave, these bays have very long residence times that can

limit the potential for dispersion and facilitate the genetic divergence of populations (e.g. 71 d in Nikko Bay, Palau; Golbuu et al. 2016). Genetically distinct populations of rainbow smelt *Osmerus mordax* have been found in bays along the northeastern coast of the USA that are geographically complex, with flow regimes that favour retention (Kovach et al. 2013). Bay scale population units have also been found in other jellyfish species. Pitt & Kingsford (2000) found significant variation in the abundance and recruitment of *C. mosaicus* in estuaries in New South Wales, Australia. This variation indicated that population regulation was occurring at the scale of individual bays, suggesting that the populations inhabiting the different bays were separate stocks (Kingsford et al. 2000, Pitt & Kingsford 2000). Further, *C. mosaicus* medusae have strong swimming abilities and could maintain positions in the upper reaches of estuaries where the advective forces are the weakest (Pitt & Kingsford 2000). These ecological data concurred with Dawson's (2005) conclusions that there were genetic differences among *C. mosaicus* populations inhabiting many of the same bays sampled by Pitt & Kingsford (2000). Similarly, great insights into the population structures of cubozoans could be gained through using genetic analyses to test clear predictions on population connectivity and spatial disjunctions that are generated from alternate methods (e.g. biophysical modelling; Dawson et al. 2005).

### Nearshore retention and patchiness

The behaviour of medusae facilitated retention near the shore and at small spatial scales (100s of m) and the medusae inhabiting these areas may be quite insular from other local populations. We demonstrated that medusae are capable of swimming to overcome nearshore current speeds, so they can maintain positions in shallow waters adjacent to beaches. Directional swimming was needed for medusae to remain non-dispersed, in groups; such groupings could only be maintained in shallow water as the current speeds and resultant dispersive forces increased with distance from shore. Fossette et al. (2015) constructed a biophysical model of the dispersion of *Rhizostoma octopus* medusae in the Bay of Biscay, France, a much more open environment than the semi-enclosed Port Musgrave. *R. octopus* medusae can form large blooms, and Fossette et al. (2015) demonstrated that the counter-current swimming of medusae facilitated the formation and maintenance of such blooms. While *C.*

*fleckeri* and *R. octopus* behave in very different ways, the importance of horizontal swimming behaviour in maintaining groupings is clear for both species.

Within estuary residency has been demonstrated (e.g. *C. mosaicus*; Pitt & Kingsford 2000), but the extreme nearshore distribution of *C. fleckeri* appears to be unusual. Immigration and emigration occurs between local populations (Kingsford & Battershill 1998) and the potential for such exchange between the east and west sides of Port Musgrave was found in this study. In many of our scenarios, however, mixing between local populations was low. Gordon & Seymour (2012) counted the rings on the statoliths of *C. fleckeri* medusae. They assumed the rings were laid down daily and calculated that the age of the oldest sampled medusae was 78 d. The model in the present study was run for 14 d in dry conditions, which is only a portion of the medusae life span. Accordingly, more mixing could occur over an entire lifespan. Additionally, there may be multiple storms in a season and, as we demonstrated, these events increase the advective forces in the bay and so increase the potential for mixing between local populations. This increased potential for mixing may not be realized if medusae utilize oceanographic refugia. There are other considerations for dispersal and connectivity. Hartwick (1991) suggested that adult *C. fleckeri* may move upstream to spawn (cubozoans are gonochoristic; Kingsford & Mooney 2014), so promoting further connectivity within an estuary, but there are no data on this.

### Dispersion potential of early life history stages

In this study, we focused on the dispersion potential of *C. fleckeri* medusae, and the evidence we provided was from medusae with interpedalial distances >4 cm. Although we did not study earlier life stages, logically they are not able to swim as well as the recorded medusae. We did model medusae as passive particles and the high retention recorded in these scenarios suggests that earlier life history stages have a limited potential for dispersion. Furthermore, 2 kinds of evidence from the literature support our conclusion of low levels of emigration: the biological characteristics of the early life stages of *C. fleckeri* and the hydrodynamics of the polyp habitat, which are characterised by structural complexity and related 'sticky water' (sensu Wolanski 1994, Andutta et al. 2012). Hartwick (1991) investigated the biological characteristics of *C. fleckeri* over the course of 11 yr.

He reared the early life stages in the laboratory and found that the zygotes and blastulae were negatively buoyant and have an adhesive coating, so they adhered to hard surfaces. These characteristics limit the amount of time that these stages spend in the water column, so reducing the potential for dispersion (Hartwick 1991). The planulae larvae emerge from the blastulae and only remain in the water column for up to 24 h before attaching to the substrate, where they metamorphose into creeping polyps (Hartwick 1991). The creeping polys become sessile, presumably after they find a suitable habitat (Hartwick 1991). Cubozoans metamorphose from polyps into nearly fully formed medusae (Werner et al. 1971), unlike the ephyrae of scyphozoans (Straehler-Pohl & Jarms 2010) which have the potential to be transported 100s of km from polyp sources to medusae sinks (Barz et al. 2006, Chen et al. 2014). As they are nearly fully formed, newly detached medusae are probably capable of swimming, and so could offer some resistance against advective currents. Indeed, newly detached *Carybdea marsupialis* medusae with diagonal bell widths as small as 0.05 cm can swim at speeds of around 1 cm s<sup>-1</sup> (C. Bordehore pers. comm.). Gordon & Seymour (2012) recorded a large maximum growth rate for *C. fleckeri* medusae of ~3 mm d<sup>-1</sup>. They constructed a Gompertz growth equation, and according to this equation a medusae could grow to 4 cm interpedalial distance (the size of the smallest individual observed in this study) in approximately 42 d. In our study, at 4 cm medusae were already capable of swimming faster than the mean current. Due to their fast growth rate, medusae probably quickly grow to be competent swimmers capable of counteracting their dispersal. Polyps are thought to reside in tidal estuaries (Hartwick 1991), although their habitat may include other coastal zones (Mooney & Kingsford 2012) these are also characterised by 'sticky water' habitats such as mangroves (Wolanski 2007). These habitats would facilitate retention due to low levels of flushing (Wolanski 2007). If juvenile medusae were flushed from the tidal estuaries they would enter Port Musgrave itself and, as demonstrated in our model of passive drift, the currents in the bay would still favour retention.

### Impact of storms on population persistence

The *C. fleckeri* medusae season overlaps with the rainy season, so medusae will be affected by pulse storm events. A strong storm event was modelled, so the flow in most events would be smaller. While the

rate of flushing increased during the storm event, most medusae remained within the system. The salinity of the shallow nearshore waters preferred by medusae remained above 21 PSU, the threshold for incapacitation identified by Mooney & Kingsford (2016b) and, even in storm conditions, medusae behaviour facilitated nearshore retention. Medusae could also find refuge in the reaches of the bay farthest from the river mouths, where waters also had salinities above 21 PSU. If the bay was stratified during river floods, then medusae could find refuge from low salinities in deeper water. Although this type of stratification is typical of estuaries (Wolanski & Elliott 2015), no data on stratification during the wet season are available for Port Musgrave. The identified and assumed refugia suggest that population persistence in Port Musgrave is highly likely during freshwater events of high impact.

#### Reliability of results from biophysical modelling

The behaviours selected for inclusion in the biophysical model were well supported by field observations and evidence from the literature (Hamner et al. 1995, Garm et al. 2007, 2011, Gordon & Seymour 2009). However, the avoidance behaviour that could further facilitate retention (where medusae perform a 180° turn when they reach mangrove habitat at the bay edges, included in the Behaviour C suite) was not necessary for the retention of medusae in the more sheltered bays on both the east and west sides of Port Musgrave, indicating that high levels of retention are likely with even simple behavioural responses. The results of the sensitivity analysis demonstrated that the modelling outputs were robust, despite major alterations to the behavioural components of the biophysical model. While considerable nearshore retention was recorded in the standard run, widening the behaviour band did improve retention. The swim speed used in the standard run was conservative given the swimming capabilities of *C. fleckeri* medusae that were discussed previously. The retention-related effects of increasing the swim speed were dependent on small scale geography (10s to 100s of m). Medusae performed the prescribed behaviours within a set distance from shore (the distance depended on the sensitivity analysis scenario). In the other direction, parallel to shore, in the bay enclosed by Box 1, the band was narrower at the bay edges than at the centre because of the concavity of the bay. Consequently, when medusae performed a 180° turn at the bay edges, they could turn into an

area beyond the behaviour band where they acted as passive particles, exaggerating the risk of loss when the swim speed was increased. This edge effect did not influence the retention of medusae in Box 2 because the bay was comparatively less concave. Future biophysical modelling studies investigating the population structure of *C. fleckeri* should consider increasing the complexity of the behavioural model to include swim speeds that vary in relation to current speeds, to avoid similar edge effects.

#### *C. fleckeri* population substructure

This study is a contribution to the growing body of evidence that *C. fleckeri* stocks are only separated by spatial scales of 10s to 100s of km. Recently, Mooney & Kingsford (2016a) found significant differences in the geochemical compositions of *C. fleckeri* statoliths from medusae collected in different regions, separated by 100s of km. Differences were even found among some sites, separated by only a few kilometres (Mooney & Kingsford 2016a). They also found that the shapes of *C. fleckeri* statoliths varied on scales of 10s to 100s of km (Mooney & Kingsford 2017).

#### CONCLUSIONS

*Chironex fleckeri* medusae were capable of swimming at velocities that greatly exceeded the current velocities in the nearshore waters where they are commonly found. The current regime and high residence time of waters in estuarine bays may assist in the retention of medusae when their swimming ability at small interpedalial distances is weak. Because few medusae may emigrate from estuarine bays, the populations within the bays may constitute stocks. The strong swimming behaviour of medusae provide evidence that medusae can maintain localised populations at spatial scales as small as 100s of m. Furthermore, the behavioural preference of medusae to remain very close to shore would allow them to survive during storm events and related freshwater pulses, where survivable salinities would be found nearshore and perhaps in deep water high salinity refugia. Our findings concur with a growing body of evidence that local populations of *C. fleckeri* may have minimal connectivity, and that stocks may often be at the scale of estuaries and bays. We predict that population genetics will reflect this conclusion.

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