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High temporal resolution sampling reveals reef fish settlement is highly clustered

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Abstract

Coral reef fish larvae settle on reefs predominantly at night around the new-moon phase, after an early developmental period spent in the pelagic environment. Most sampling is conducted across whole nights, and any studies that have examined the frequency of arrival within nights have typically been limited to coarse sampling time scales of 1–5 hours. Here, we present results for arrival numbers of fish caught between dusk and midnight from light traps sampled every 15 min at an Indonesian coral reef, providing the finest temporal resolution for this type of study to date. A Spatial Analysis by Distance IndicEs (SADIE) analysis, adapted to temporal data, revealed clustering of reef arrival times for many species, with an increase in catches immediately after dusk dropping off towards midnight. Importantly, the timing of clusters differed among species indicating that different factors determine the timing of arrival among taxa. Our results support the hypothesis that larval behaviour influences the timing of arrival at a coral reef for different fish species.

Keywords: Coral reefs; fish larvae; larval behaviour; larval settlement; SADIE analysis; Indo-Pacific.

Introduction

Most coral reef fish spend their early life stage as larvae in the open ocean before returning to reefs to settle (Montgomery *et al.* 2001; Kingsford *et al.* 2002; Leis and McCormick 2002). Settlement stage larvae move onto the reef predominantly at night (Robertson *et al.* 1988; Stobutzki and Bellwood 1998), with a few species potentially also settling to the reef during the daytime (e.g. Dufour and Galzin 1993; Kingsford 2001). Settlement is higher around the new and third quarter moon, when moonlight is weak (Milicich 1988; Meekan *et al.* 1993; Milicich and Doherty 1994; Sponaugle and Cowen 1997; Leis and Carson-Ewart 1999; D'Alessandro *et al.* 2007;). Nocturnal settlement has been suggested as a life strategy for avoidance of predators feeding on fish larvae at the reef (Hamner *et al.* 1988), which could otherwise have a significant impact on the already high mortality rate of settlement stage fishes (Almany and Webster 2006; Dytham and Simpson 2007). The high risk of mortality typical of this life stage of demersal reef fishes is also thought to drive other behaviours in larval fish including auditory and olfactory orientation (Atema *et al.* 2002; Simpson *et al.* 2004, 2005) and schooling (Pitcher 1986; Leis 2006; Codling *et al.* 2007; Simpson *et al.* 2013; Irisson *et al.* 2015).

Because of the small size of larval fish and their tendency to settle during low-light levels, it is challenging to directly observe larval behaviour *in situ*, which often has to be inferred from controlled environments (e.g. olfactory preference Dixson *et al.* 2008; swimming speed Fisher and Bellwood 2003) or *in situ* observations made during the daytime (Dixson *et al.* 2014; Simpson *et al.* 2005; Leis *et al.* 2015). From nocturnal sampling methods that are highly restricted in space and time it should be possible to make informed speculations on the behaviours that drive settlement patterns. However, only a few studies have examined the arrival patterns of fish over single night periods (Dufour *et al.* 1996; McIlwain 1997; Wilson 2003), with the highest temporal resolution being used typically in the range of one recording every 1–2 hours. Whilst this can provide us with useful information on when the main settlement peaks occur during the night, it is not sufficient temporal resolution to capture arrival of individual cohorts of fish. In the present study we assessed arrival of fish larvae to Indonesian reefs using light traps sampled every 15 min: a finer temporal resolution than any previous sampling method used in this context. The light trap sampling technique is stage selective, capturing

phototactic larval species at a competent settlement stage in high numbers. It does have species sampling biases dependent on the degree of phototactic response and the swimming abilities of the individual larvae (Choat *et al.* 1993). Overall, however, this technique lends itself well to the study of larval settlement, providing large catches and ease of replication (Milichich 1988; Choat *et al.* 1993; Wilson 2001, 2003). The focus of this study was not to examine the arrival rate of settlement stage fish for this region (which would also be of interest), as it does not cover lunar, seasonal, or yearly temporal variation, but to explore at high temporal resolution the arrival of fish within single nights.

We hypothesised that: a) settlement stage fish would not arrive at a constant rate in the light traps but would display a high degree of clustering in the catches due to behaviour influencing settlement times; and b) that the arrival rate of different species would follow different arrival patterns if hydrodynamic forces were not the main drivers of clustering patterns.

Materials and Methods

Sites

Sampling was carried out at two sites offshore from the coral reefs of Hoga Island, Southeast Sulawesi, Indonesia (Fig.1), selected based on their different water flow regimes. Buoy 1 is a site located on the reef in the channel between Hoga Island and Kaledupa Island through which a large body mass of water moves daily with the tides. Buoy 5 is located off the western reef of the island and is relatively sheltered although the large changes in tidal range (~2m) can result in relatively strong water currents during tide flows towards and away from the shore. There were differences in the acoustic composition of the reef sound spectra and some of the coral composition of the reef (Piercy 2015), but no other known significant differences between them.

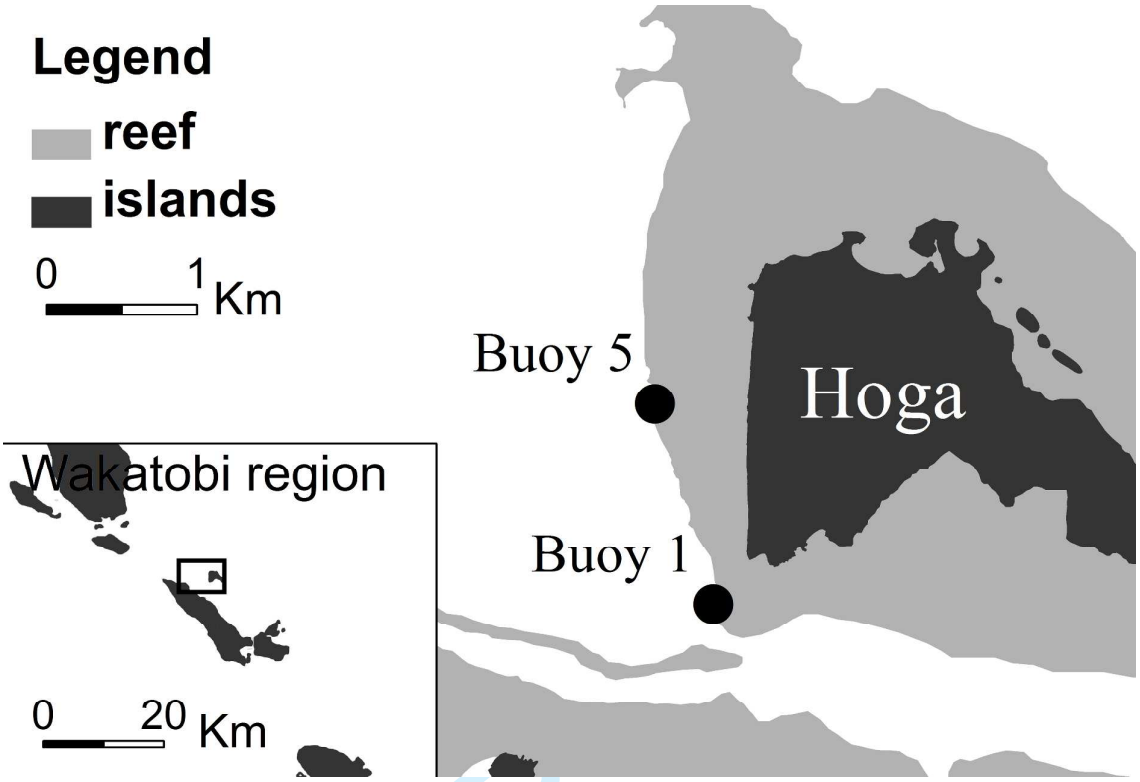

Figure 1. Sites of light trap sampling off Hoga Island, Indonesia (5° 28' 20" S; 123° 45' 25" E). Buoy 1 is located in the channel between Hoga Island and Kaledupa (bottom left corner of this map) where the water current is strongest. Buoy 5 is located in a central position along the Hoga Island western reef and is dominated by inshore and offshore currents as the reef flat (45º grey bars) fills and drains.

Sampling

Sampling was carried out between the third quarter moon, 24 July 2011, and three days after the new moon, 3 August 2011 (new moon was 31 July 2011) between dusk and midnight (18:00–00:00). The focus of this study was on collecting high temporal resolution data rather than lunar or seasonal changes that have been previously extensively examined (e.g., Robertson *et al.* 1988; Stobutzki and Bellwood 1998; Dufour and Galzin 1993; Kingsford 2001). This "snapshot" approach is representative of a typical new moon recruitment period but may differ in terms of abundance and species composition compared to other times of the year. Throughout the night time period, light levels remained low as the moon had not yet risen with the exception of the last two sampling dates (1

and 3 August) when the moon did not set until 19:30 and 21:00 respectively. However, considering the proximity of these dates to the new moon phase the maximum fraction of the moon illuminated was 17.8% (3 August) with the moon at a maximum altitude of 40° (18:15 2011-08-03) estimated using the MoonAngle function in R (package oce, version 0.9-20), with half or more of the sampling time being undertaken in the absence of any moonlight. Buoy 1 was sampled on nights of the 24 July 2011, and 1 and 3 August 2011, while B5 was sampled on 26, 28 and 30 July 2011.

Sampling was conducted using two Stobutzki and Bellwood (1997) light traps, suspended 1 m below the water surface and fishing alternatively every 15 min throughout the sampling periods. The traps were fitted with a 40 W 12 V fluorescent tube light connected to a 12 V 12 Ah lead acid battery charged to >13 V. A boat was moored to a buoy to which the light trap was attached during deployment at 1.5 m below the sea surface. The boat was attached to the mooring using 30 m of rope to maintain it at a distance from the light trap and mooring and to minimise disturbance to recruiting fish. Every 15 min the boat was hauled closer to the first light trap, and the second light trap was deployed immediately prior to the first one being extracted from the water to maintain a constant light source in the water column. The contents of the first light trap were emptied via a funnel into a 10x10x10 cm container with mesh netting openings on the sides and sealed with a mesh netting cap. The operation was carried out within a 20 L bucket containing fresh seawater 10 cm deep to maintain the fish alive and re-capture any escaped fish during the emptying operation. The container was then placed in a fish mesh holder over the side of the boat. Once all 24 samples over the night period had been collected, the mesh containers were placed inside a 100 L polystyrene container filled with fresh seawater, brought back to the shore, and kept aerated using two airstones. The next day, fish from each container were sedated using a mixture of clove oil, 90% ethanol, and sea water in a ratio of 1:3:6 respectively (see ethical note in Simpson *et al.* 2008). The fish from each container were spread out on a gridded tray with 1 cm of water and photographed lying on their side using a Samsung S860 8.1 MP digital camera (Samsung Electronics Co., Ltd., Suwon, South Korea) and allowed to recover in a 50 L aerated plastic tank containing fresh seawater before being released back onto the reef at dusk. Fish were identified from the photos to family level using the guide by Leis and Carson-Ewart

(2000), and to genera and species where feasible. Fish that were difficult to identify were preserved in 70% ethanol after being anaesthetised and photographed using a Veho VMS-1 USB microscope with x200 maximum magnification (Veho Electronics, Hampshire, UK). All work was carried out under permits held by D. Smith and issued by the Indonesian Minister for Research and Development.

Clustering analysis

To test for non-randomness and provide indices for the degree of clustering on the temporal distribution of fish counts, the Spatial Analysis by Distance IndicEs (SADIE) methodology (Perry *et al.* 1999) was applied using the SADIEShell program (Open Source under GNU General Public License, V3). This analysis is usually used to test for spatial clustering of species in a two-dimensional grid, but can also be applied to one-dimensional data sets such as quadrats positioned in line along a transect (Perry *et al.* 2002). Since the calculation of the indices is based on a "spatial" matrix of count data, to transpose this problem to a one-dimensional (1-D) context an artificial extra dimension was included where units on the x axis were represented by the times of sampling and their position on the y axis was assumed to be a constant (i.e. y = 1; Perry, pers. comm.). Hence, for I = 1,…, n cells were of the form (x_i, 1), each containing an observed sample count. This method calculates three indices for the counts of data before testing them against random permutations of the counts to provide the probability *p* of the measures of aggregation against the randomised ones (Perry 1998; Perry *et al.* 1999).The first measure is the *index of aggregation* (Perry 1998), which quantifies the degree of effort required for each cell to reach an even distribution across all cells. The index of aggregation, *Ia*, is defined as:

$$Ia = D/Ea \quad (1)$$

where *D* is the distance to regularity, defined as the minimum value of the total distance that the *ith* individual sample unit at position (x_i, 1) would have to move, from one unit to the next, so that all units contained an identical count. *Ea* is the arithmetic mean distance to regularity for the randomised samples.

The second index is the *distance to crowding index* (Perry 1998), a measure of the minimum effort required for the counts of each cell to move into a single cell. The distance to crowding index, J_a, is defined as

$$J_a = F_a/C \quad (2)$$

where C is the distance to crowding, defined as the minimum value of the total distance that the ith individual sample unit at position (x_i, 1) must move so that all are congregated in one unit. F_a is the arithmetic mean distance to crowding for the randomised samples. This index increases in value as the distance to crowding C decreases, (i.e. a high index value indicates a more clustered group, with a lower distance needed for all data to move to the same sampling point). The index is more powerful at detecting a cluster than the index of aggregation I_a but cannot be interpreted correctly if more than one cluster is present.

The final measure is the *degree of clustering*. Similar to the distance to regularity, it calculates the effort required for each cell to reach an even distribution among the cells. However, in this case it computes the degree to which each data point influences the overall clustering, by calculating the strength of inflow and outflow from one cell to another in order to reach an even count between cells. For donor unit i, at position (x_i , 1), the outflow to the j of n_i receiver units, $j = 1, \ldots, n_i$, at position (x_j , 1), is denoted as v_{ij} . The distance of this flow d_{ij} is

$$d_{ij} = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2} = |x_i - x_j| \quad (3)$$

The average distance of outflow from unit i, weighted by the magnitude of each individual flow, is denoted as Y_i, where $Y_i = \Sigma_j d_{ij} v_{ij} / \Sigma_j v_{ij}$. The same calculation is carried out for inflows.

The distances for inflows and outflows need to be standardised in order to obtain the dimensionless clustering indices. For outflows, a standardised and dimensionless index of clustering, v_i, is then given by:

$$v_i = Y_i \, {}_oY / {}_iY \, {}_cY \quad (4)$$

where Y_i is the distance of the flow; ${}_iY$ is the expected value of the average absolute flow distance for the *i* unit, assuming a random arrangement of the observed counts among the observed sample units, where the outflows are computed for each count that is randomly assigned to the *i* Unit. Similarly, instead of following the unit *i,* through the counts randomised to it, we can follow the count *c* through its randomisations to different units, where ${}_cY$ is the expected value of the average flow distance for the observed count *c*; ${}_0Y$ is the expected value of the overall average absolute distance of flow for all points and counts in the randomisations. By convention, inflows are given negative scores and outflows positive scores and the average of their absolute values is used as the clustering index. Only taxa for which at least an average of one individual per sampling time was collected on any one day (i.e. > 24 individuals) were included in the analysis.

Association analysis

Temporal association between the arrival rates of different fish taxa was measured using the SADIE association index. This method first calculates the similarity between the clustering indices of the two populations at each time point then averages those similarities to provide an overall measure of association: the correlation coefficient.

The measure of local spatial association for unit *i* is given by:

$$\chi_i = \frac{n(z_{i1} - q_1)(z_{i2} - q_2)}{\sqrt{\sum_k (z_{i1} - q_1)^2 \sum_k (z_{i2} - q_2)^2}} \quad (5)$$

where z_{k1} denotes the clustering indices of the first set of data, with mean q_1, and z_{k2} denotes the clustering indices for the second set of data, with mean q_2. The overall spatial association is the mean of these local values:

$$X = \Sigma_i \chi_i / n \quad (6)$$

where X is the correlation coefficient between the clustering indices of each set. The correlation is not performed directly on the counts because large count values would contribute disproportionately to the correlation coefficient. The significance of the association or disassociation is calculated by permutation of the counts. Since the randomisation process produces a histogram with the probability

for both association and disassociation, the α value to accept or reject the null hypothesis is divided between the two ends of the distribution. Thus $\alpha = 0.05$ is split between the two extremities so that the null hypothesis is rejected either for $p > 0.975$ (significant disassociation) or $p < 0.025$ (significant association).

Autocorrelation is a property present in all clustering scenarios and could increase the chance of finding non-existent associations and dis-associations because of the lack of independence of the samples from one another. To minimise this effect and as recommended in Perry *et al.* (2002), a Dutilleul adjustment was applied to account for the degree of autocorrelation and reduce the effective sample size (Dutilleul 1993). To do this, where necessary, the sets were detrended, and the degrees of freedom corrected for correlation, *M*-2, where *M* is the effective sample size. The critical values are inflated by a scale factor of $\sqrt{\frac{M-3}{n-3}}$ and the significance of the randomisation test adjusted accordingly. An extreme example of this would be if all samples are so strongly autocorrelated that we only need one sample in order to estimate the size of all the remaining samples, effectively reducing the sample size to one.

Results

During the 144 light trap deployments and collections a total of 2,187 individual fish were caught, representing 28 families, of which some Pomacentridae, Apogonidae, and Sygnathidae could be identified to genus level and even separated into species. The species themselves could rarely be identified due to lack of guides on species level identification for fish at the larval stage for this region of high biodiversity; therefore some species may have been grouped under the same taxon.

No fish were caught during the first sampling period on any night (18:00–18:15) and only one individual fish from the genus *Spratelloides* was caught on the second sampling period (18:15–18:30) over the six sampling days. More fish were caught at B1 (1,656) compared to B5 (531 individuals; Chi-square test, $\chi^2 = 37.8$, $p<0.001$, d.f. = 2).

Clustering

All taxa that met the inclusion criteria on at least one day (>24 individuals caught on that sampling night), except Gobiidae and Synodontidae, displayed significant temporal clusters for at least one of the measures for clustering (degree of clustering, index of aggregation and distance to crowding; Table 1 and Figs. 2-5). The significance level of the degree of clustering and the index of aggregation was in agreement for all taxa (i.e. they were either both significant or both non-significant). However, the significance level of the distance to crowding index occasionally differed from the other two indices. A significant clustering effect was observed in *Corythoichthys* sp.1 (family Sygnathidae) on 24 July 2011, *Abudefduf* sp.1 (family Pomacentridae) on 1 August 2011, and in *Apogon* sp.1 (family Apogonidae), *Apogon* sp.3, *Chromis sp.* (family Pomacentridae) on 3 August 2011 according to the distance to crowding but not the degree of clustering or the index of aggregation (Table 1 and Figs. 2 and 3). This is likely due to the greater sensitivity of the former index compared to the latter indices when a single cluster is present. In contrast, the Gobiidae were significantly clustered according to the degree of clustering and the index of aggregation on 30 July 2011 and 1 August 2011, but not according to the distance to crowding index. This is likely due to the fact that the latter index only provides meaningful results in the presence of a single cluster in the data, whilst two or three temporal clusters appear to be present in Gobiidae catches on those days (Figs. 5a, b).

Table 1. Clustering indices from the SADIE methodology for fish taxa that met the minimum catch requirement (minimum 24 fish of the taxa caught over the sampling night). Significant clustering indices are highlighted in bold with the significance level in brackets below (underlined).

Species or nearest identifiable taxon	Site	Day	# individuals	Degree of clustering (p)	Index of aggregation (p)	Distance to crowding index (p)
Apogon sp. 1	Buoy 1	2011-08-03	40	1.70 (0.069)	1.72 (0.063)	**1.90 (0.001)**
Apogon sp. 2	Buoy 1	2011-08-03	48	**1.73 (0.012)**	**1.87 (0.032)**	**2.11 (0.001)**
Apogon sp. 3	Buoy 1	2011-08-01	41	**1.99 (0.032)**	**1.93 (0.034)**	**2.11 (<0.001)**
		2011-08-03	52	1.44 (0.143)	1.59 (0.090)	**1.82 (0.003)**
Chromis sp.	Buoy 1	2011-07-24	245	**2.18 (0.015)**	**2.29 (0.010)**	**1.64 (<0.001)**
		2011-08-01	133	**1.97 (0.035)**	**2.07 (0.022)**	**1.87 (<0.001)**
		2011-08-03	133	1.57 (0.097)	1.68 (0.072)	**1.76 (<0.001)**
Gobiidae	Buoy 1	2011-08-01	42	**1.99 (0.025)**	**1.92 (0.026)**	1.20 (0.209)
		2011-08-03	27	0.75 (0.685)	0.77 (0.654)	1.18 (0.173)
	Buoy 5	2011-07-26	63	1.39 (0.101)	1.30 (0.140)	0.96 (0.611)
		2011-07-28	38	1.45 (0.128)	1.58 (0.091)	1.39 (0.061)
		2011-07-30	50	**1.90 (0.021)**	**1.80 (0.035)**	1.36 (0.178)
Holocentridae	Buoy 1	2011-07-24	31	**2.68 (0.002)**	**2.52 (0.002)**	**2.80 (<0.001)**
Pomacentrus sp. 1	Buoy 1	2011-07-24	29	**2.47 (0.005)**	**2.78 (0.001)**	**1.81 (0.001)**
Corythoichthys sp.1	Buoy 1	2011-07-24	105	1.38 (0.140)	1.30 (0.180)	**2.51 (0.001)**
Synodontidae	Buoy 1	2011-07-24	31	0.45 (0.999)	0.51 (0.994)	0.69 (0.920)
Abudefduf sp.1	Buoy 1	2011-07-24	248	**1.742 (0.060)**	**1.93 (0.033)**	**2.10 (<0.001)**
		2011-08-01	53	**2.12 (0.022)**	**2.30 (0.011)**	**2.17 (<0.001)**
	Buoy 5	2011-07-26	27	1.23 (0.233)	1.23 (0.235)	**1.49 (0.011)**

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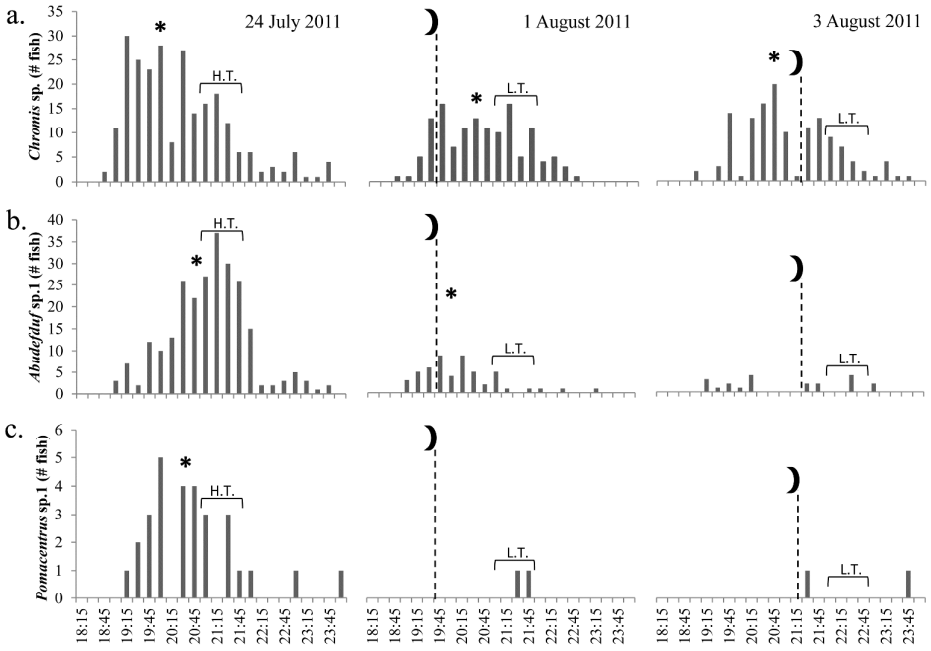

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242 Figure 2. Arrival numbers of different Pomacentrid taxa at Buoy 1 on three different nights. a) Arrival
243 numbers of *Chromis sp.,* the most abundant taxon; b) *Abudefduf* sp.1*,* the second most abundant
244 taxon; and c) *Pomacentrus* sp.1. The scales for the fish counts vary between panels. The time period
245 of the high (H.T.) and low (L.T) tides are indicated above their respective periods and the time of the
246 moon set is indicated with a dashed line and moon symbol. For 24 July there was no moon in the sky
247 during the sampling period. Nights on which a significant temporal cluster in arrival numbers was
248 present for at least one of the clustering indices presented in Table 1 are indicated by an asterisk (*).

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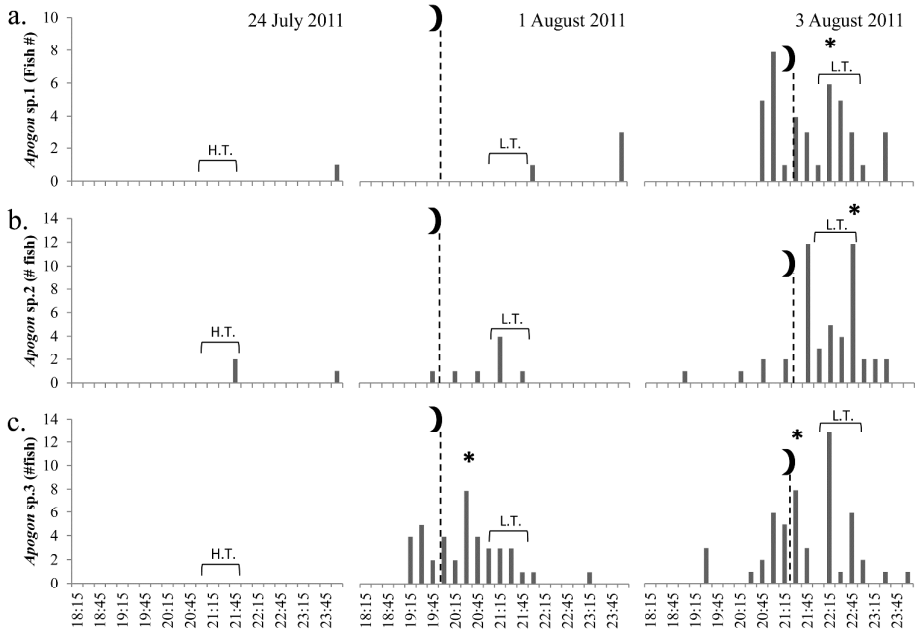

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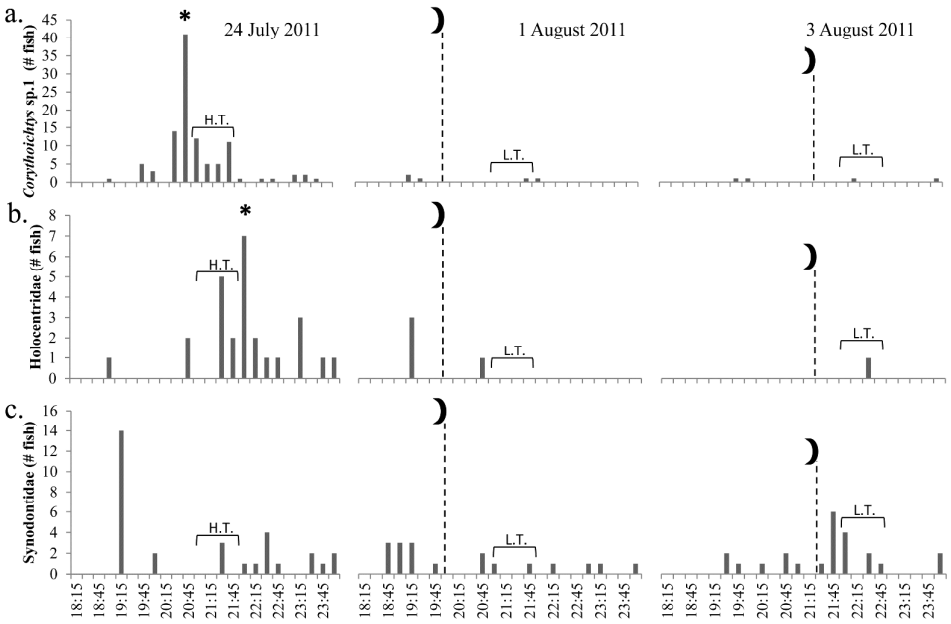
251 Figure 3. Arrival numbers of different Apogonid taxa at Buoy 1 on three different nights. a) Arrival

252 numbers of *Apogon* sp.1*;* b) *Apogon* sp.2; and c) *Apogon* sp.3. Explanation of the symbols is given in

253 Fig. 2.



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255 Figure 4. Arrival numbers of selected taxa at Buoy 1 on three different nights. The taxa presented all

256 met the selection criteria of >24 individuals on a single sampling night: a) *Chorythoichtys* sp.1

257 (Family: Sygnathidae); b) Holocentridae; and c) Synodontidae. Explanation of the symbols is given in

258 Fig. 2

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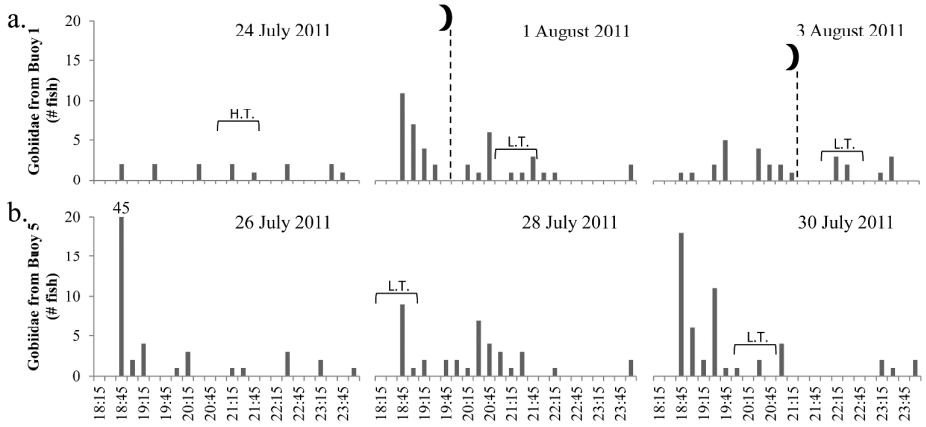

Figure 5. Arrival numbers on six different nights of Gobiidae at: a) Buoy 1; and b) Buoy 5. Explanation of the symbols is given in Fig. 2. Where >20 individuals were caught at a single sample point, the number of fish caught is indicated above the bar.

Associations

There were significant associations in the arrival numbers among taxa of the same family (e.g. Pomacentridae: *Chromis sp.* associated with *Abudefduf* sp.1 and *Pomacentrus* sp.1, and Apogonidae: *Apogon* sp.1 associated with *Apogon* sp.3), but also between taxa from different families (e.g. *Chromis sp*. associated with *Apogon* sp.1, and *Chromis sp.* associated with Gobiidae; Table 2). Most association analyses between taxa only met the inclusion criteria for one of the sampling days (both taxa with >24 individuals caught on the day). Exceptions to this were comparisons between *Chromis sp.* and *Abudefduf* sp.1 (significantly associated on both days; X= 0.45, p = 0.02, and X = 0.68, p < 0.001 on 24 July 2011 and 1 August 2011 respectively), and *Chromis sp.* and Gobiidae, which were significantly associated on 3 August 2011 (X = 0.44, p = 0.02) but not on 1 August 2011, when the Gobiidae arrived in more than one cluster (Fig. 6).

Table 2. Association indices between temporal arrival numbers of different fish taxa. The comparisons are made only between taxa on days for which both had a total catch of >24 individuals (i.e. average of >1 individual per sampling period). The symbol N/A denotes where no comparisons were possible because one species in each pair had fewer than 24 individuals. The date is indicated in

the top line of the box, followed by the association index below with its significance level and Dutilleul adjusted sample size in brackets. Significant associations are highlighted in bold.

	Chromis sp.	*Apogon* sp.1	*Apogon* sp.2	*Apogon* sp.3	*Abudefduf* sp.1	*Pomacentrus* sp.1	*Corythoichthys* sp.1	*Synodontidae*	*Holocentridae*	*Significant associations per testable pairs*
Chromis sp.	-	-	-	-	-	-	-	-	-	4 / 9
Apogon sp. *1*	2011-08-03 0.37 (0.092; 24)	-	-	-	-	-	-	-	-	1 / 4
Apogon sp.2	2011-08-03 0.13 (0.56; 21.7)	2011-08-03 0.35 (0.14, 22.9)	-	-	-	-	-	-	-	0 / 4
Apogon sp.3	**2011-08-01 0.71 (<0.001, 24)**	**2011-08-03 0.69 (0.004, 19.8)**	2011-08-03 0.36 (0.16, 19.8)	-	-	-	-	-	-	2 / 4
Abudefduf sp.1	**2011-07-24 0.45 (0.04, 22.8)** **2011-08-01 0.68 (<0.001, 22.9)**	N/A	N/A	N/A	-	-	-	-	-	1 / 6
Pomacentrus sp.1	**2011-07-24 0.45 (0.04, 22.4)**	N/A	N/A	N/A	2011-07-24 –0.12 (0.60, 23.7)	-	-	-	-	1 / 5
Corythoichthys sp.1	2011-07-24 0.26 (0.32, 24)	N/A	N/A	N/A	2011-07-24 0.54 (0.14, 11.1)	2011-07-24 0.01 (0.8, 21.7)	-	-	-	0 / 5
Synodontidae	2011-07-24 0.33 (0.24, 21.2)	N/A	N/A	N/A	2011-07-24- 0.10 (0.64, 21.6)	2011-07-24 –0.29 (0.08, 20.9)	2011-07-24 –0.18 (0.20, 21.7)	-	-	0 / 5
Holocentridae	2011-07-24 0.33 (0.16, 23.8)	N/A	N/A	N/A	2011-07-24 0.58 (0.06, 13)	2011-07-24 0.04 (0.82, 23.3)	2011-07-24 0.45 (0.12, 18.5)	2011-07-24 –0.21 (0.14, 23.1)	-	0 / 5
Gobiidae	2011-08-01 –0.01 (0.98, 19.8) **2011-08-03 0.44 (0.04, 23.9)**	2011-08-03 0.35 (0.14, 22.9)	2011-08-03 –0.14 (0.62, 20.3)	2011-08-01 0.002 (0.90, 24) 2011-08-03 0.15 (0.52, 18.6)	2011-07-26 –0.16 (0.72, 20.2) 2011-08-01 0.001 (0.90, 19.3)	N/A	N/A	N/A	N/A	1 / 5

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Discussion

To our knowledge, this is the first study to present high temporal resolution arrival numbers of settlement stage fish. In this study clear temporal clustering was widespread among the ten most abundant taxa over single night sampling periods, usually involving a single cluster for taxa identified to genus level. Importantly, the arrival pattern in the light traps across the night often differed among taxa, which suggests that different mechanisms could underlie their settlement timing. For the taxa identified Pomacentridae taxa, 82-90% were caught between 19:00 and 22:00 on all sampling days at Buoy 1, while the three taxa from the next most abundant family, Apogonidae, arrived later in the evening, with 80-85% caught between 21:00 and 23:00 on 3 August but earlier for *Apogonidae* sp.3 on 1 August (19:00 – 22:00).

The fact that the Gobiidae were present in higher numbers at the site with lower flow could be a strategy to increase their chances of settlement. Indeed, their smaller size compared to other taxa collected is likely to result in slower swimming speeds (reviewed in Leis 2010). This would make active swimming more energetically demanding due to the higher viscosity environment faced by smaller fish (Bellwood and Fisher 2001) and the higher water flow, hence diminishing their settlement success. Although larvae from the Gobiidae family did not appear to arrive at the traps in clusters, this could be a result of the low taxonomic identification level. In fact, an outlier in Fig. 5b where 45 individuals (39% of the total Gobiidae caught on that sampling night) were caught in a single 15 min sampling period, hints at possible high levels of clustering in a narrow time frame. A possible explanation for this high catch could be that the Gobiidae were shoaling, as observed in laboratory and field studies for larvae in this family (Breitburg 1989; Breitburg 1991). This may also be true for the arrival of Synodontidae, which displayed no significant pattern of settlement but did recruit in high numbers on a single sampling period on 24 July (Fig. 4c). However, these conclusions remain speculative.

Comparisons among species forms an important part of this study. Setting aside taxa identified only to family level, where lack of associations might be due to multiple species composition of the catches, the arrival patterns of *Apogon* sp.2 and *Chorythoichthys* sp.1 were not similar to any of the other taxa.

There was also a lack of association between arrival patterns of two Pomacentrid species, *Abudefduf* sp.1 and *Pomacentrus* sp.1.

Settlement stage larvae of *Chromis* sp. have been observed to settle in small groups during the daytime (Nolan 1975). *Chromis atripectorialis* improve orientation consistency, are able to more accurately maintain a bearing, and swim faster when they school compared to movement as individuals (Irisson *et al.* 2015). This could provide an important survival advantage to larvae that school as this behaviour would enable them to reach a settlement site faster, therefore reducing the time spent in the pelagic environment where they incur high mortality rates (Houde and Zastroe 1993). Persistent aggregations have been found to occur in the splitnose rockfish, *Sebastes diploproa,* where 11.6% of settlement stage larvae were siblings (Ottmann *et al.* 2016). Interactions among group members may play a role in maintaining these aggregations from spawning to settlement and could provide the basis for clustering patterns observed in this study. It is important to note, however, that there are a number of other mechanisms which could drive the observed clustering patterns, including concentration of larvae in particular locations due to mesoscale eddies (Shulzitski *et al.* 2016). It is also unclear how larvae would be able to maintain a school at night without being able to use vision to see other group members, although other senses such as lateral line sensing (Faucher *et al.* 2010) or vocal communication (Staaterman *et al.* 2014) may play a role.

There are insufficient sampling days in this study to determine whether different arrival patterns observed among taxa are explained by random arrival of patches of larvae or driven by behaviours aimed at improving settlement for a particular species. Why different taxa have different patterns of arrival could, for example, be due to a trade-off between finding limited suitable settlement habitat and avoiding predators. For *Corythoichthys* sp.1, that is widespread around the island and settle in seagrass habitat between the reef crest and the shore (pers. obs.), avoidance of predators may be a more important factor than limited habitat availability compared to species that recruit to specific coral types like many pomacentrids. If this were the case, it might explain why they recruit only later in the night when no light is available to predators (Johannes 1978; Dytham and Simpson 2007), and the narrower time frame over which the recruitment occurred in this study (Fig.4a). The latter could

increase their chances of passing through the "wall of mouths" (Hamner et al. 1988) awaiting them at the reef by achieving safety in numbers according to group theory (Bertram 1978).

This study furthers our understanding of the manner in which settlement stage fish recruit to the reef in this critical transition phase, but cannot explain why different species display clustering patterns, whether group behaviour mediates the temporal clustering or how and when the clustering is initiated. Aggregations of larvae prior to settlement have been documented (Patterson *et al.* 2005), however, whether this is due to environmental processes (e.g. eddies) that concentrate larvae in particular locations or whether behaviour mediates the observed clustering patterns, will require further studies that directly observe larvae during this critical life period..

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