

1 Temporal patterns of spawning and hatching in a spawning aggregation of the
2 temperate reef fish *Chromis hypsilepis* (Pomacentridae)¹

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4 William Gladstone

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6 School of Environmental and Life Sciences, University of Newcastle, PO Box 127,

7

Ourimbah NSW 2258, Australia

8

9 E-mail: William.Gladstone@newcastle.edu.au

10

Tel.: + 61-2-43484123

11

Fax: + 61-2-43484145

12

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13 **Abstract** Descriptions of temporal patterns in the reproduction of damselfishes
14 (family Pomacentridae) and adaptive hypotheses for these patterns are derived mostly
15 from studies of coral reef species. It is unclear whether the types of temporal patterns
16 and the explanatory power of the adaptive hypotheses are applicable to damselfishes
17 of temperate rocky reefs. This study tested hypotheses about the existence of lunar
18 spawning cycles, the diel timing of hatching, and the synchronization of temporal
19 patterns in hatching and tides in the schooling planktivorous damselfish *Chromis*
20 *hypsilepis* on a rocky reef in New South Wales, Australia. Reproductive behaviour
21 was observed daily for 223 d between August 2004 and March 2005. *C. hypsilepis*
22 formed large spawning aggregations of 3,575–33,075 individuals. Spawning occurred
23 at a uniform rate throughout the day on a semi-lunar cycle. The greatest number of
24 spawnings occurred 1 d after the new moon and 1 d before the full moon. The cost to
25 males from brood care was an 85% reduction in their feeding rate. The semi-lunar
26 spawning cycle may be an outcome of the use of the lunar cue to synchronize the
27 aggregation for spawning of widely dispersed individuals and the need for males to
28 recuperate after brooding. Eggs hatched 3-7 hr after sunset following a 4.5 d
29 incubation period. This study provides no support for hypotheses that link temporal
30 patterns in hatching with particular tidal regimes believed to facilitate early survival
31 of larvae and their dispersal. The result that hatching occurred over the tidal cycle was
32 due to the rapid off-reef dispersal of larvae from the spawning ground at all stages of
33 the tide. *C. hypsilepis* is similar to other planktivorous damselfishes in its semi-lunar
34 spawning cycle, cost of brood care, and protracted diel spawning regime. It differs in
35 its lengthy period of hatching and its breeding in spawning aggregations, believed to
36 be rare among demersally spawning fishes.

37

38 **Introduction**

39

40 Reproduction by damselfishes (family Pomacentridae) involves demersal spawning in
41 a prepared nest site, a period of broodcare by the male parent, and a pelagic larval
42 phase (Thresher 1984; Asoh and Yoshikawa 2002). Descriptions of temporal patterns
43 in damselfish reproduction have mostly focused on three aspects: lunar cycles of
44 spawning within the reproductive season; diel patterns in spawning; and the timing of
45 hatching (Keenleyside 1972; Pressley 1980; Doherty 1983; Thresher 1984; Foster
46 1987; Gladstone and Westoby 1988; Kohda 1988; Robertson et al. 1990; Alcala and
47 Sikkell 1994; Goulet 1995; Sikkell 1995; Tzioumis and Kingsford 1995; Mizushima et
48 al. 2000; Asoh and Yoshikawa 2002; Asoh 2003). Spawning by most damselfishes
49 occurs on a lunar or semi-lunar cycle (Gladstone and Westoby 1988; Asoh 2003).
50 Adaptive explanations for these cycles invoke benefits to: (1) the hatching propagules
51 (such as facilitated off-reef dispersal from synchronization with spring tides, predator
52 swamping, and reduced intraspecific competition for food); (2) the spawning adults
53 (such as migration to the spawning site and crepuscular spawning itself being
54 facilitated by moonlight); or (3) the brooding males (such as cooperative defense
55 against predators and the opportunity to recover from the energetic costs associated
56 with territory defense and broodcare) (Robertson et al. 1990; Robertson 1991).

57

58 The larvae of damselfish hatch 1–2 hr after sunset following a 3–5 d
59 incubation period (Doherty 1983; Thresher 1984; Gladstone and Westoby 1988).
60 Various authors have suggested that reproductive success will be increased if larvae
61 hatch on a falling tide, especially the spring tide, and are rapidly removed from reef-
62 based predators and/or more widely dispersed (Johannes 1978; Lobel 1978; Ross
63 1978; Barlow 1981; Doherty 1983; Robertson 1983; Gladstone and Westoby 1988).
64 The potential benefits to larvae from hatching on a falling tide has been one of the
65 most widely invoked explanations for the temporal patterns in hatching, despite the
66 fact that not all damselfish larvae hatch on a falling tide and that the predicted benefits
67 have not been demonstrated (Shapiro et al. 1988; Robertson et al. 1990).

68

69 An understanding of the patterns of temporal variation in damselfish
70 reproduction and the associated adaptive hypotheses has been developed largely from
71 studies of coral reef species. Reviews of the reproduction of damselfishes indicate that

72 the temporal patterns of reproduction are known only for a limited number of
73 temperate reef damselfishes. Gladstone and Westoby (1988) summarized data on 26
74 species of tropical damselfishes. Robertson et al. (1990) studied 15 species of tropical
75 damselfishes. Tzioumis and Kingsford (1995) reviewed information on the
76 reproductive behaviour of four species of temperate damselfishes and 31 species of
77 tropical or sub-tropical species. Asoh (2002) summarized the spawning parameters of
78 30 species of tropical and sub-tropical damselfishes. Since the review of Tzioumis and
79 Kingsford (1995) information on the spawning of only one further temperate reef
80 species has been published (Picciulin et al. 2004). Factors that potentially influence
81 reproductive strategies of fishes, including biology (e.g. body size, diel behaviour
82 patterns, prevalence of predators) and environment (e.g. productivity, seasonality,
83 shelter availability), differ between tropical and temperate regions (Ebeling and Hixon
84 1991). The generality of the explanatory models for damselfish reproduction is
85 therefore potentially limited by this focus on tropical species.

86

87 *Chromis hypsilepis* (Günther, 1876) is a planktivorous damselfish that feeds in
88 large schools over rocky reefs in south-east Australia, Lord Howe Island, Norfolk
89 Island, and northern New Zealand. It attains a maximum length of 150 mm (Kuitert
90 2000). *C. hypsilepis* is especially abundant on the rocky reefs of central-southern New
91 South Wales (personal observations). Although reported to spawn with no predictable
92 frequency (Tzioumis and Kingsford 1995) there are no detailed studies of its
93 reproduction. Evidence from other planktivorous damselfishes (Gladstone and
94 Westoby 1988; Robertson et al. 1990; Tzioumis and Kingsford 1995; Asoh and
95 Yoshikawa 2002) suggests that *C. hypsilepis* is likely to be a colonial spawner on a
96 lunar or semi-lunar cycle with males brooding demersal eggs that hatch just after
97 sunset. Accordingly, this study tested the following hypotheses: (1) *C. hypsilepis*
98 spawns on a lunar cycle; (2) the eggs of *C. hypsilepis* hatch within 2 hr of sunset; and
99 (3) temporal patterns in hatching coincide with the falling tide.

100

101 **Materials and methods**

102

103 Study area

104

105 This study was carried out at Terrigal reef, New South Wales, Australia (33°27'00"S,
106 151°26'00"E) (Fig. 1). Terrigal reef fringes a coastal headland for a distance of 2.2 km
107 and consists of 4 habitats: a shallow algal-dominated fringe at 3-5 m; barren boulder
108 reef slope with abundant sea urchins (*Centrostephanus rodgersii*) from 5–18 m; deep
109 reef from 18–22 m (where the reef edge met sandy bottom) dominated by encrusting
110 life forms (e.g. sponges, ascidians, corals); and stands of kelp (*Ecklonia radiata*)
111 interspersed between barren boulder and deep reef habitats at 10–15 m depth. This
112 mix of habitats is typical of the coast of New South Wales (Underwood et al. 1991).
113 Preliminary observations indicated that *Chromis hypsilepis* at Terrigal reef spawned
114 only in the barren boulder habitat in a small section of the reef at its western extremity
115 at 7–10 m depth (hereafter called the spawning ground). The tidal regime at Terrigal
116 reef is semi-diurnal tidal with approximately 2 high tides and 2 low tides occurring
117 over a 24 hr period.

118

119 Field observations of spawning

120

121 Daily surveys were undertaken (weather permitting) between 1 August 2004 and 11
122 March 2005. No prior information was available on the spawning seasonality of *C.*
123 *hypsilepis* although preliminary surveys by the author had established that spawning
124 did not occur during the period March-September 2003. Therefore the study period
125 was selected to determine the specific duration of the spawning season. A 50 m fixed
126 transect (identified by regularly spaced, small sub-surface floats) was established in
127 the middle of the spawning ground at the beginning of the study. On each daily census
128 of the fixed transect the following variables were recorded: (1) number of adult *C.*
129 *hypsilepis* occurring in a 1 m band to one side of the transect and within 1 m of the
130 substratum and engaged in reproductive activities (defending territories, inspecting
131 territories, courtship, egg care); (2) number of spawnings. Daily censuses were done
132 over a 30 min interval between 0800 and 1000 h. The number of spawnings that
133 occurred on days when observations were not possible because of poor weather was
134 estimated by back-calculating from the number of egg clutches recorded, the last date
135 these egg clutches were observed, and a 4.5 d incubation period (see Results). This
136 was only necessary for days 49-55 (Fig. 2a).

137

138 The days on which spawnings occurred were converted to a day in a 29 d lunar
139 cycle where the new moon occurred on day 1 and the full moon occurred on day 15.
140 The total number of spawnings that occurred on each day of the lunar cycle was
141 calculated and converted to a percentage of total spawnings. Rayleigh's test (Zar
142 1999) was used to test whether spawning frequency was uniformly distributed
143 throughout the lunar cycle. The existence of distinct cycles of spawning was
144 examined by autocorrelation analysis. The data set of the number of spawnings
145 observed daily over 136 d (from the start of spawning on 1 October 2004 to the last
146 day on which spawning was observed, 12 February 2005) was made stationary by
147 first-order differencing prior to analysis (Chatfield 1996).

148

149 Diel variation in the frequency of spawning was quantified from the number of
150 spawnings observed in the fixed transect over a 30 min period on a single day at 0600
151 h, 0830 h, 1130 h and 1500 h in each of 3 consecutive spawning cycles. Observations
152 were done in 2005 on 10 January, 22 January and 9 February. Sunrise occurred at
153 approximately 0500 h during this period. The number of spawnings was standardized
154 to the number of spawnings 1000 adult⁻¹ *C. hypsilepis* present in the fixed transect so
155 that variations in the size of the spawning aggregation between cycles did not obscure
156 diel differences in spawning frequency.

157

158 Cost of brooding

159

160 Feeding rate was quantified in January 2004 as a measure of the potential costs
161 associated with brood care by males and as a possible explanatory factor for the
162 observed spawning periodicity (Robertson et al. 1990). The feeding rate of *C.*
163 *hypsilepis* was quantified as the number of bites min⁻¹. The feeding rate of *C.*
164 *hypsilepis* that were not engaged in egg care was determined while scuba diving with
165 a school as they fed in the water column (at 2–5 m depth) and separately observed 10
166 individuals for a period of 5 mins each. The number of bites observed during that time
167 was recorded. As *C. hypsilepis* are planktivorous it was not possible to observe
168 individual prey items being consumed. It was therefore assumed that a biting action in
169 the water column indicated a successful feeding event. It was also not possible to
170 determine the sex of individuals as they fed in the water column. It was therefore
171 assumed that the feeding rate did not differ between males and females outside the

172 reproductive periods. The feeding rate of males that were engaged in egg care (four
173 days after spawning) was quantified by separately observing 10 individuals for 5 mins
174 each while scuba diving and recording the number of bites in the water column. The
175 null hypothesis of no difference in the feeding rates of individuals not engaged in egg
176 care and males engaged in egg care was tested by a *t*-test.

177

178 Length of incubation and diel timing of hatching

179

180 The length of the incubation period was determined by monitoring all egg clutches in
181 the transect from the day they were laid until the day after they hatched during a
182 spawning cycle in October 2004 and January 2005. The position of individual egg
183 clutches was noted in relation to their distance along the fixed transect and prominent
184 substrate features, to allow daily monitoring of their development. Diel timing of
185 hatching was determined by collecting a sample of eggs from each of 6 clutches on
186 the day they were due to hatch and placing each in separate 500 mL plastic jars
187 immersed within aerated 75 L aquaria in the laboratory. Eggs were observed hourly
188 from 1600 h (3 hr before sunset) and larvae present at each hourly census were
189 siphoned from the plastic jars until hatching was completed.

190

191 The daily records of spawning frequency were lagged by 4.5 d (the incubation
192 period) to produce a daily record of hatching frequency. This method was used as an
193 indicator of temporal variation in hatching in preference to daily measurements of the
194 size of egg clutches because the latter would have accidentally disturbed or damaged
195 nearby nests because of the high density of brooding males in the spawning
196 aggregation site. The experiment on diel hatching times provided information on the
197 starting time and duration of hatching (3 hr after sunset and 4 hr respectively, see
198 Results). The state of the tide during hatching was determined from the times of high
199 and low tides in tables of the predicted tides for Sydney Harbour
200 (<http://www.waterways.nsw.gov.au/>), with no lag observed between the 2 locations.
201 The hypothesis that hatching occurred on a falling tide was tested by overlaying the
202 daily record of hatching frequency with the record of days on which high tide
203 occurred during the hatching period. A coincidence of the 2 sets of records would
204 indicate that hatching only occurred on days when high tide occurred 3–7 hr after
205 sunset. A more detailed test of an association between hatching and tide was done by

206 determining the stages of the tide that occurred over the course of hatching of each
207 clutch. The observed frequency distribution of hatching times (in units of hours before
208 and after high tide) was compared to an expected distribution of hatching times (of the
209 same total number of hatchings) that would occur if hatching was restricted to the
210 falling tide. The expected distribution of hatching times was assumed to be a left-
211 skewed distribution with the largest frequencies of hatching occurring midway
212 between high and low tides and no hatchings occurring on the rising tide. The
213 observed and expected distributions were compared by the Kolmogorov-Smirnov test
214 (Sokal and Rohlf 1995). The hypothesis that the numbers of clutches that hatched
215 peaked on days of spring tide was tested by cross correlation analysis. At significant
216 correlation at a lag of zero days would support the hypothesis. The number of clutches
217 that hatched daily was equal to the number of observed spawnings lagged by the
218 incubation period (4.5 d). The daily maximum in tidal height was obtained from the
219 predicted tides for Sydney Harbour.

220

221 **Results**

222

223 General observations

224

225 Male *Chromis hypsilepis* established territories at the spawning ground from 6
226 September 2004 and the numbers of fish increased daily thereafter (Fig. 2a). Females
227 traveled to the spawning ground in lines of about 100 individuals and upon entering
228 the spawning ground began examining males' territories. Spawning occurred on a
229 rock surface within the male's territory. Although not quantified, females spawned
230 with more than 1 male on a single day and males spawned with more than 1 female on
231 a single day. Egg clutches were 15–30 cm diameter. Females left the spawning ground
232 after spawning and males remained in their territory defending egg clutches until
233 hatching. Males left the spawning ground after their eggs hatched. The only exception
234 to this occurred in October 2004 during the first spawning cycle of the season when
235 males remained on the spawning ground to feed and defend their territories until the
236 next spawning cycle began.

237

238 There was considerable variation in the size of the spawning aggregation over
239 the spawning season (Fig. 2a). The maximum density of males and females that

240 aggregated in a spawning cycle varied from 143 to 1,323 fish 50 m⁻² (n=9 cycles).
241 Scaling upwards to the total area of the spawning ground suggests that the total
242 number of adult *C. hypsilepis* that aggregated varied between 3,575 and 33,075.

243

244 Spawning cyclicity

245

246 Nine spawning cycles were observed in the 2004/05 spawning season (Fig. 2b).
247 Spawning began on 1 October 2004 (water temperature 18⁰C). The last eggs hatched
248 from the final spawning cycle on 16 February 2005 (water temperature 21.5⁰C).
249 Therefore the spawning season lasted from 1 October 2004-16 February 2005, a total
250 of 139 d. The duration of spawning cycles (i.e. number of days between first
251 spawning and completion of egg hatching) was 6-13 d (mean±SE=9.0±0.8 d).
252 Spawning continued for 2-9 d (5.0±0.8 d) within a spawning cycle. The interval
253 between successive spawning cycles was 5-12 d (8.2±1.0 d). A total of 875 spawnings
254 were observed in the fixed transect in 2004/05. The daily spawning frequency (i.e. the
255 number of spawnings observed during the daily survey) was significantly correlated
256 with the total number of *C. hypsilepis* in the fixed transect ($R_s=0.58$, $P<0.001$, $n=39$,
257 significance determined by randomization because of lack of independence).

258

259 Plots of the daily sizes of spawning aggregations (Fig. 2a) and the daily
260 spawning frequency (Fig. 2b) suggest that peaks in both occurred around the times of
261 the new and full moons. Spawning frequency peaked within 1-3 d of the new and full
262 moons, except for the first two spawning events when spawning peaked 5 d after the
263 new moon and 4 d after the full moon respectively. The semi-lunar spawning cycle
264 was confirmed when all spawnings were standardized to a day of the lunar month
265 (Fig. 3). Rayleigh's *Z*-statistic found that spawnings were not uniformly distributed
266 throughout the lunar cycle ($Z = 75.98$, $P < 0.001$). Autocorrelation analysis revealed
267 significant correlations at lags of 2 d and 12 d (Fig. 4). The significant 12 d
268 autocorrelation supports the separation of peak spawning frequencies in Fig. 3. The
269 significant 2 d autocorrelation indicates a short-term correlation in the numbers of *C.*
270 *hypsilepis* spawning.

271

272 Diel variation in spawning

273

274 Comparison of mean spawning frequency throughout the day by analysis of variance
275 showed that, although spawning frequency gradually declined throughout the day, this
276 diel variation was not significant (Fig. 5). Therefore the frequency of spawnings
277 recorded in the daily censuses between 0800 and 1000 h was likely to be a reliable
278 indicator of the maximum daily spawning frequency.

279

280 Cost of brooding

281

282 In non-reproductive periods *C. hypsilepis* fed from 2–3 m above the substratum to 0.5
283 m below the surface. Feeding bouts (when biting occurred almost continuously) lasted
284 1-3 min with a bite rate of 36.1 ± 1.45 bites min^{-1} (mean \pm SE, $n=10$) (range: 25-42).
285 Male *C. hypsilepis* guarding clutches of eggs fed in bouts during which they swam
286 upwards to about 1 m above their clutch, bit at plankton for no longer than 2-3 sec,
287 then returned to their clutch. The average bite rate of males tending a clutch was
288 5.4 ± 1.45 bites min^{-1} (range: 1-13). The two feeding rates were significantly different
289 ($t=14.99$, $P<0.001$).

290

291 Hatching

292

293 Daily monitoring of individually identified egg clutches produced from recorded
294 spawnings showed that egg clutches were absent on the fifth morning after spawning
295 had been observed. This indicates an incubation period of 4.5 d. This did not appear to
296 change throughout the spawning season, despite the change in water temperature from
297 18°C to 21.5°C . Eggs progressed in appearance from a light pink coloured mat on the
298 same day of spawning to dark grey on the day of hatching with the eyes of embryos
299 clearly visible as silver spots. Frequently the edge of egg clutches began to peel away
300 from the rock on the day of hatching. In the aquarium experiment it was found that
301 eggs began hatching at 2200 h (3 hr after sunset) and no further larvae hatched after
302 0200 h (7 hr after sunset). On the night of the experiment high tide occurred at 2230 h
303 and therefore most larvae hatched that night on a falling tide. However, this pattern
304 was not repeated for all hatchings over the spawning season. A visual comparison of
305 the daily record of hatching frequency and the record of days when high tide occurred

306 3 – 7 hr after sunset (Fig. 6) shows that most hatchings occurred close to or coincided
307 with high tide only in cycles 3, 4, 6, 7 and 8. This pattern is confirmed upon
308 examination of the state of the tide that occurred during the course of hatching of each
309 clutch (Fig. 7). Clutches hatched at all stages of the tidal cycle, with the greatest
310 frequency of hatchings occurring 1 hr before and 1 hr after high tide. The observed
311 and expected frequency distributions of hatchings over the tidal cycle were
312 significantly different ($D=165.08$, $P<0.001$).

313

314 Peaks in the number of clutches hatching coincided with spring tides for 2
315 spawning cycles (cycles 3 and 8) and coincided with, or were near to, ebb tides for 6
316 spawning cycles (cycles 1, 2, 5, 6, 7 and 9) (Fig. 6). The cross-correlation factor for a
317 lag of zero days (0.14) was not significant (Fig. 8), which indicated that the number of
318 clutches that hatched on a day was not correlated with the daily maximal tidal height.

319

320 **Discussion**

321

322 *Chromis hypsilepis* spawned on a semi-lunar cycle, with the greatest number of
323 spawnings occurring 1 d after the new moon and 1 d before the full moon. This result
324 contrasts with the observation of Tzioumis and Kingsford (1995) that *C. hypsilepis* at
325 other locations spawned with no predictable cycling frequency. Spawning periodicity
326 has been reported for many species of damselfishes with lunar, semi-lunar or periodic
327 cycling more common than acyclic spawning (Thresher 1984; Ochi 1986; Gladstone
328 and Westoby 1988; Robertson et al. 1990; Robertson 1991; Tzioumis and Kingsford
329 1995; Mizushima et al. 2000; Asoh and Yoshikawa 2002; McIlwain 2002; Asoh
330 2003; Picciulin et al. 2004). Ecological hypotheses proposed to explain spawning
331 periodicity invoke benefits to either the larval offspring (via enhanced off-reef
332 dispersal, predator saturation, tracking of larval food, reduced competition, or
333 synchronization with ideal settlement periods) or the adults (via moonlight facilitating
334 migration and/or spawning, saturation of egg predators, enhanced predator defence, or
335 reductions in the overall cost of paternal care to males) (Allen 1972; Keenleyside
336 1972; Johannes 1978; Colin et al. 1987; Gladstone and Westoby 1988; Foster 1989;
337 Robertson et al. 1990; Gladstone 1994; Petersen and Warner 2002).

338

339 In attempting to provide an ecological explanation for the semi-lunar
340 spawning cycle of *C. hypsilepis* it is possible to eliminate hypotheses relating to the
341 use of moonlight for migration, pre-dawn spawning, and facilitated egg care.
342 Migration to the spawning ground and egg care occurred in both the low light (i.e.
343 near new moon) and high light (i.e. near full moon) phases of the lunar cycle. This is
344 evident in Fig. 4 when the spawning data is lagged by the incubation period of 4.5 d.
345 Spawning also occurred during daylight hours. In addition, hypotheses relating to
346 saturation of predators of larvae, eggs, and brooding males are also unlikely
347 explanations for the periodicity of spawning by *C. hypsilepis*. The variable amounts of
348 time that spawning occurred for in each spawning cycle (range of 2–9 d) meant that
349 hatching, and numbers of brooding males present at the spawning site, were not
350 highly synchronous. A high degree of synchronicity is required for these hypotheses
351 to be true (Robertson et al. 1990).

352

353 I suggest that the semi-lunar cycling of reproduction in *C. hypsilepis* is a
354 response to two factors: (1) the costs of reproduction; and (2) the need for a cue to
355 synchronize the gathering of widely dispersed individuals for spawning. Male *C.*
356 *hypsilepis* incurred a cost associated with their egg care, measured as a reduction in
357 feeding rate of 85%. This is comparable to, although higher than, the costs recorded
358 for other planktivorous, egg-brooding damselfishes (Robertson et al. 1990). Female *C.*
359 *hypsilepis* also incur a cost from reproducing via their production of eggs and travel to
360 the spawning aggregation site. Cycling would therefore provide an opportunity for
361 both male and female *C. hypsilepis* to recover from their reproductive efforts.
362 However, the need for brooding males to recuperate after each spawning cycle cannot
363 be the sole explanation for the observed semi-lunar periodicity. A simple periodic
364 cycle unrelated to the lunar cycle could provide that recovery (e.g. *Chromis dispilus* in
365 Tzioumis and Kingsford 1995). *C. hypsilepis* is a schooling planktivore. Feeding
366 schools were observed at different locations around the reef's perimeter (a total length
367 of 2.2 km) and it is likely that some individuals migrated over this total distance to
368 reach the spawning ground. Schools of *C. hypsilepis* occasionally fed over the
369 spawning ground (personal observations), although the schools were never of the
370 same size as the aggregations observed in the spawning ground. It is therefore
371 unlikely that aggregations (of the size observed in this study) formed as a result of
372 social cues provided by a small number of males that established mating territories

373 (Robertson 1991). I suggest that the semi-lunar spawning cyclicality reflects the use of
374 lunar cues by *C. hypsilepis* to facilitate the aggregation of widely dispersed
375 individuals, and also allows males to recuperate between spawning cycles.

376

377 Contrary to the proposed hypothesis, the eggs of *C. hypsilepis* did not hatch
378 only during a falling tide. Hatching occurred on all stages of the tidal cycle and peaks
379 in hatching did not coincide with the spring tide. The existence of a link between
380 hatching and the tidal cycle has been tested frequently in studies of reef fish
381 reproductive ecology (Johannes 1978; Ross 1978; Thresher 1984; Gladstone and
382 Westoby 1988; Robertson et al. 1990; Robertson 1991; Gladstone 1994; Tzioumis and
383 Kingsford 1995; Sancho et al. 2000; McIlwain 2002). The basis of this hypothesis is
384 the assumption that larvae that hatched on a falling tide would be carried rapidly away
385 from reef-based predators and would suffer less mortality. Although this adaptive
386 explanation is appealing, the association between hatching and falling tides has been
387 confirmed for some species of damselfishes (Ross 1978; Kingsford 1985; Robertson
388 et al. 1990; Mizushima et al. 2000; McIlwain 2002) but not others (Ochi 1986;
389 Robertson et al. 1990; Tzioumis and Kingsford 1995).

390

391 The most likely explanation for the temporal patterns in hatching of *C.*
392 *hypsilepis* eggs is that tidal phase was irrelevant to the likelihood of larval dispersal
393 and survival. A parallel study (Gladstone unpublished data) found that dispersal was
394 much more rapid from the spawning site, compared to non-spawning sites, regardless
395 of the tidal phase. Therefore, properties of the spawning site used by *C. hypsilepis* (i.e.
396 rapid off-reef dispersal regardless of tidal phase) explain the absence of a close
397 association between hatching frequency and tidal phase.

398

399 *C. hypsilepis* spawned throughout the day and there was little change in
400 spawning frequency. This observation is consistent with the results of other studies
401 that have found that planktivorous damselfishes that engage in colonial nesting spawn
402 throughout the day (Thresher 1984; Gladstone and Westoby 1988; Robertson et al.
403 1990; Asoh and Yoshikawa 2002; Asoh 2003). Permanently territorial damselfishes
404 spawn over a narrow time frame around dawn (Doherty 1983; Thresher 1984;
405 Gladstone and Westoby 1988; Robertson et al. 1990) but see (Sikkel 1995). Thresher
406 (1984) argued that spawning time may be optimized so that larvae hatch at the

407 optimal time for dispersal, and most damselfishes, except *C. hypsilepis*, hatch over a
408 very short time period just after sunset (Doherty 1983; Thresher 1984; Foster 1987;
409 Gladstone and Westoby 1988; Robertson et al. 1990; Alcala and Sikkell 1994; Asoh
410 and Yoshikawa 2002). The adaptive basis for the extended diel spawning period of
411 colonially nesting damselfishes has not been investigated in detail (Petersen and
412 Warner 2002).

413

414 The extended diel spawning period of *C. hypsilepis* is likely to be a result of
415 factors relating to the timing of hatching and social behaviour. Hatching of *C.*
416 *hypsilepis* eggs was not confined to a particular tidal phase and, therefore, the timing
417 of spawning was not critical. The extended period of diel spawning is reflected in the
418 extended period of nocturnal hatching and the lack of tidal controls to hatching. A
419 social factor that is likely to contribute to the existence of an extended diel spawning
420 period is the protracted period of female arrivals at the spawning site. Females
421 continued to arrive at the spawning site throughout the day (personal observations).
422 The protracted period of arrival is probably due to the distance over which females
423 had to migrate to reach the spawning site and intrinsic physiological differences
424 between individual females in their response to the lunar cue for spawning. It is likely
425 that the cost to males from defending additional eggs in their nest (arising from
426 continued spawnings) is not excessive. Therefore, males are able to accept additional
427 spawnings throughout the day. If true, there is no impediment to spawning continuing
428 throughout the day.

429

430 The large aggregation of *C. hypsilepis* that formed on a semi-lunar cycle to
431 spawn and brood eggs can be defined as a 'spawning aggregation' (Domeier and
432 Colin 1997; Claydon 2004). The features of *C. hypsilepis*' reproduction that allow it
433 to be categorized as a spawning aggregation include migration away from the normal
434 feeding grounds to form temporary aggregations for spawning; use of the same
435 location by the aggregation over successive spawning cycles within a spawning
436 season and over successive spawning seasons; and temporal predictability. Although
437 not documented here, the spawning aggregation was observed by the author in the
438 same location in 2003/2004 and 2005/2006 and local divers have known about its
439 existence for 25 years (L Graham personal communication). There was considerable
440 variation in the size of spawning aggregations throughout the spawning season (Fig

441 3a). Kingsford (1980) attributed variations in the size of spawning groups of *Chromis*
442 *dispilus* to variations in local weather conditions. This is unlikely to be the sole reason
443 for *C. hypsilepis* because large variations in the size of the spawning aggregation
444 occurred over similar weather conditions (e.g. the last 3 cycles in Fig 2a). *C. hypsilepis*
445 is unusual amongst species forming spawning aggregations because of its small size
446 and demersal spawning habit. Only one other species with a demersal spawning habit
447 (*Pseudobalistes flavimarginatus*, Balistidae) has been documented to spawn in
448 aggregations (Gladstone 1994) and pelagic spawning has been hypothesized as a
449 prerequisite to the formation of spawning aggregations. Pomacentrids that nest
450 colonially are not recognized as aggregative spawners because of the absence of a
451 migration from the normal feeding grounds to a distinct spawning site (Claydon
452 2004). The spawning aggregation of *C. hypsilepis* is likely to be a useful model for
453 testing general hypotheses about the processes and factors underlying the formation of
454 spawning aggregations that may be more difficult to test in other, larger species.

455

456 **Conclusion**

457

458 The temporal patterns of reproduction in *C. hypsilepis* support predictions that
459 schooling, planktivorous damselfishes will spawn colonially on a lunar or semi-lunar
460 cycle over a protracted period of the day. The use of a lunar cue by *C. hypsilepis* to
461 synchronize the gathering of widely dispersed individuals and to allow males to
462 recuperate from their brooding duties lends further support to the usefulness of adult-
463 biology hypotheses (Robertson et al. 1990) to explain temporal patterns in
464 reproduction. The lack of an association between temporal variation in hatching and
465 the tidal cycle suggests that local factors (in this case the water movements from the
466 spawning ground) can explain the observed temporal patterns in hatching.

467

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474

475 **References**

476

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581

FIGURE CAPTIONS

Fig. 1 *Chromis hypsilepis*. Location of study site.

Fig. 2 *Chromis hypsilepis*. Diel variation in spawning frequency (number of spawnings 1000 adult⁻¹ *Chromis hypsilepis*). Values shown are mean number of spawnings \pm SE (n = 3 cycles). The difference between time intervals is not significant (one-way analysis of variance $F_{3,8} = 0.46$, $P = 0.71$, untransformed data, Cochran's C = 0.62 $P > 0.05$).

Fig. 3 *Chromis hypsilepis*. Temporal variation in (a) size of spawning aggregations (no. fish 50 m⁻²) and (b) frequency of spawnings (recorded as no. spawnings observed 50 m⁻² during the daily surveys). Daily results are shown for 1 September 2004 (day 1) to 24 February 2005 (day 179). Daily surveys actually began on 1 August 2004 and concluded on 11 March 2005 but no reproductive behaviours were observed before 1 September 2004 or after 24 February 2005.

Fig. 4 *Chromis hypsilepis*. Number of spawnings observed on each day of the lunar month as a % of total spawnings (day 1 is the new moon). ● = new moon. ○ = full moon.

Fig. 5 *Chromis hypsilepis*. Correlogram of autocorrelation coefficients for daily spawning frequency. Correlation coefficients exceeding the 95% confidence limits (dashed line) are significant (Chatfield 1996).

Fig. 6 *Chromis hypsilepis*. The number of egg masses that hatched throughout the 2004/2005 spawning season in relation to the maximum daily tidal height. Peaks in tidal height represent spring tides and troughs represent ebb tides. Daily results are shown for 1 September 2004 (day 1) to 24 February 2005 (day 179). The number of egg masses that hatched on each day was the number of spawnings lagged by the incubation period of 5 d. Boxes enclose days on which high tide occurred 3-7 hr after sunset (i.e. times of hatching).

Fig. 7 *Chromis hypsilepis*. The tidal stages that occurred over the course of each egg clutch's hatching (observed values). Stages of the tidal cycle are represented in units of 1 hr before (negative values) and 1 hr after (positive values) high tide. It has been assumed that the 875 clutches hatched over 875 x 4 hour-long intervals. The expected distribution of tidal stages was simulated by distributing the same number of hour-long hatching intervals over the high-low tide cycle with maximal hatching occurring midway through the falling tide.

Fig. 8 *Chromis hypsilepis*. Cross-correlation factors (CCF) between number of egg masses that hatched on a day and the maximum daily tidal height for lags of 0 to 7 days. The dashed lines are the 95% confidence limits.













