

## The contrasting hatching patterns and larval growth of two sympatric clingfishes inferred by otolith microstructure analysis

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**Abstract.** Larval abundance, age, growth and hatching patterns of two sympatric clingfishes, *Gobiesox marmoratus* and *Sicyases sanguineus* (Pisces, Gobiesocidae), were estimated by using otolith microstructure analysis and compared on the basis of collections performed during the austral spring in 2010 off the coast of central Chile. *G. marmoratus* larvae were more abundant than *S. sanguineus* larvae during the study period. For both species, the sagittae deposited micro-increments during embryonic development (before hatching) and a hatch mark was observable in all examined otoliths. The sagittae otoliths of *G. marmoratus* grew in radius, perimeter and area faster than did the otoliths of *S. sanguineus*. Both species showed significant ( $P < 0.05$ ) differences in larval growth and lunar periodicity of the hatching events. *G. marmoratus* hatched at smaller sizes (2.6 mm) mainly during the first-quarter moon and the larvae grew at rates of  $0.24 \pm 0.01 \text{ mm day}^{-1}$ . *S. sanguineus* hatched as larger larvae ( $> 3 \text{ mm}$ ) during the first-quarter and full moons and grew at slower rates ( $0.14 \pm 0.01 \text{ mm day}^{-1}$ ) during the initial 25 days. The high abundance of larval clingfish in near-shore waters, temporal decoupling among the hatching events, and the different growth rates may be tactics to increase self-recruitment in coastal waters.

**Additional keywords:** Gobiesocidae, *Gobiesox marmoratus*, lunar cycle, sagittae, *Sicyases sanguineus*.

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### Introduction

The timing of spawning in synchrony with the lunar cycle, either at a new or full moon, has been reported in several demersal spawners (Johannes 1978; Robertson *et al.* 1990, 1999; Courtney *et al.* 1996; Yamahira 1997; Takemura *et al.* 2004). These studies suggested that the new moon provides suitable conditions for survival because darkness would reduce the predation pressures on spawned eggs, whereas moonlight can be advantageous when adults migrate to spawning sites (Robertson *et al.* 1990, 1999). For example, certain territorial and site-attached damselfishes exhibit reproductive activity that peaks near the new and/or full moon. Eastern king prawns show two periods of increased spawning activity during the lunar cycle (Courtney *et al.* 1996). The advantage of these strategies may be to minimise immediate egg and larval predation and to facilitate their transportation to offshore locations by the strongest outgoing

tide (Takemura *et al.* 2004). Alternatively, such a reproductive pattern might be controlled on the basis of parental interests, for example, such as through a reduction in predation risk on the parents or enhanced efficiency in their feeding, mating or parental activity (Okuda and Ohnishi 2001).

However, spawning that occurs on a semi-lunar cycle, either in synchrony with a new full-moon period or associated with the quarter-moon phases reported in several studies, is more controversial (Noichi *et al.* 1994; McIlwain 2002; Sponaugle and Pinkard 2004; Gladstone 2007). Some authors have suggested that spawning can be continuous over the lunar cycle and larvae derived from eggs spawned during either the spring or neap tides would have a better chance of survival (Noichi *et al.* 1994; Sponaugle and Pinkard 2004), which is presumably linked to an adaptive strategy to enhance offshore dispersal or larval retention, respectively. Furthermore, the semi-lunar spawning

patterns could be a result of a strategy to spawn in synchrony with the tidal cycle because neap tides are coupled with quarter moons and spring tides are coupled with full moons, which has been reported in the settlement patterns of several demersal fishes (Sponaugle and Cowen 1994; Robertson *et al.* 1999; Plaza-Pastén *et al.* 2003).

In the past two decades, several studies have addressed the link between hatching patterns and the lunar cycle, by using otolith microstructure analysis (Sponaugle and Cowen 1994; Noichi *et al.* 1994; Plaza-Pastén *et al.* 2003; Sponaugle and Pinkard 2004). This technique obtains precise estimations of the hatch date by subtracting the number of daily rings from the capture date. In addition, estimations of age and growth can also be obtained and compared with hatch patterns. However, most otolith-based studies of hatch patterns have remained restricted to either tropical areas or temperate zones in the northern hemisphere. Conversely, studies on lunar spawning synchrony in the southern hemisphere are scarce (but see McIlwain 2002) and remain non-existent in some areas. This is true for the south-eastern Pacific Ocean where several families of inter-tidal fishes occur (e.g. Blennidae, Labrisomidae, Clinidae, Gobiidae, Gobiessocidae). Members of these families are demersal spawners, a reproductive mode that has been traditionally viewed as a distinct, alternative strategy to maximise larval survival and return to the adult population (Sponaugle and Cowen 1994). One of the most common groups in intertidal and subtidal zones of central Chile, south Pacific, is the family Gobiessocidae, which is ecologically important in these types of environment.

Clingfishes are small goby-like fishes and have fused pelvic fins that form a sucker, a single spineless dorsal fin, a scaleless head and body, genital papilla behind the anus, and no swim bladder (Nelson 2006). The Gobiessocidae family includes 43 genera and ~150 species distributed in shallow waters along tropical and temperate seashores (Nelson 2006). This family occupies a variety of microhabitats, such as boulder fields, exposed rocky substrates, coarse gravels, empty bivalve shells, the stems and bulbs of kelp and seagrass beds (Cancino and Castilla 1988; Gonçalves *et al.* 2002). The adults lay demersal egg clusters attached to rocks. The adults perform fanning, mouthing and guarding of the eggs as forms of parental care (Pires and Gibran 2011; Tojeira *et al.* 2012). The embryonic development may occur from 12 to 24 days (Ruck 1971, 1973; Pérez 1981; Tojeira *et al.* 2012). The larvae are well developed at hatching and have a small yolk sac, functional jaws, fully pigmented eyes and a body pigment similar to that of the post-yolk sac larvae. The size at hatching ranges from 2.4 to 6.8 mm (Allen 1979; Pérez 1981; Leis and Rennis 2000).

Along the south-eastern Pacific coast of central Chile, three species of gobiessocids are observed; *Gobiesox marmoratus* Jenyns, 1842, *Sicyases sanguineus* Muller & Troschel, 1843, and *Tomicodon chilensis* Brisout de Barneville, 1846, are common under the boulders of rocky shores (Pequeño 1989). *G. marmoratus* inhabits boulder fields (Pérez 1981) and has carnivorous habits, mainly preying on amphipods, crustacean decapods, molluscs and fish (even juvenile *S. sanguineus*) (Muñoz and Ojeda 1998; Pardo-Gandarillas *et al.* 2004). *S. sanguineus* attains a larger size (30 cm) than does *G. marmoratus* (~9 cm), has osmotic adaptations for dehydration during emersion (Marusic *et al.* 1981) and migrates from the highest point in

the inter-tidal zone, where it consumes inter-tidal animals and seaweed as a juvenile, to the low inter-tidal and subtidal waters, where it becomes an adult (Cancino and Castilla 1988). This species is also consumed by humans (Loot *et al.* 2005). The smallsucker clingfish, *Tomicodon chilensis*, is distributed from northern Peru to central Chile and is an omnivorous feeder, mainly preying on macro-algae and gastropods (Berrios and Vargas 2004).

The eggs and larvae have been described for *G. marmoratus* and *S. sanguineus* (Pérez 1981), on the basis of spontaneous spawning in the laboratory; however, the larval growth rates and early life ecology are currently unknown. The main goal of this research was to estimate and compare the larval growth and hatching patterns of two clingfish species from central Chile, namely, *G. marmoratus* and *S. sanguineus*, on the basis of the analysis of otolith microstructure.

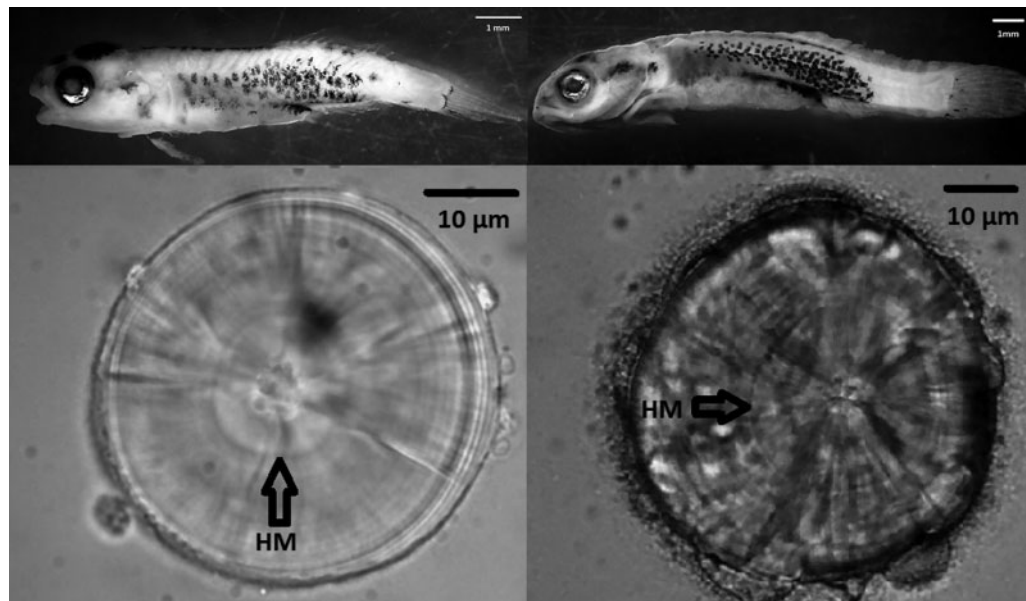
## Materials and methods

### Fieldwork

During the late austral winter and spring of 2010, three dusk and nocturnal coastal surveys (from 1930 hours to 2300 hours) were conducted in El Quisco Bay (33°24'S, 71°43'W) on board the RV Ilán. Casts of a conductivity, temperature and depth (CTD) with a Seabird SBE-19 were performed at each station from the surface to 30 m deep, and oblique hauls of a Bongo net (60-cm diameter, 300- $\mu$ m mesh size) with two TSK flowmeters (Tsurumi-Seiki Co., Ltd., Yokohama, Japan) mounted in the frame of the net were performed for 15–20 min from a depth of 20 m. Seawater filtered by the net ranged from 34.1 to 316.4 m<sup>3</sup> (mean  $\pm$  one s.d.: 201.5  $\pm$  76.5 m<sup>3</sup>), and varied according to the relative abundance of zooplankton. Subsequently, the nets were washed on board and all zooplankton samples ( $n = 38$ ) were initially fixed with 5% formalin buffered with sodium borate and preserved in 96% ethanol after 12 h. Although there are no formal studies about reproduction seasonality of both species, the period of study was selected during a season when abundance of larval stages of both species is conspicuous and larger than in other season (Hernández-Miranda *et al.* 2003).

### Laboratory work

In the laboratory, all larval fish were separated, counted and identified into the lowest possible taxon. Clingfish larvae of the species *G. marmoratus* and *S. sanguineus* were identified on the basis of the criteria described by Pérez (1981) and separated into pre- and post-flexion stages (flexion and post-flexion were pooled together) (Fig. 1). Larval abundance was standardised to individuals (ind.) per 1000 m<sup>3</sup>, utilising the flowmeter counts. The following three measurements to the nearest 0.01 mm were taken on each larva under an Olympus SZ-61 stereomicroscope (Olympus America Inc., Tokio, Japan) with a Moticom 2500 (5.0M Pixel, Motic Group Co., Ltd. Xiamen, China) video camera connected to a PC with the Moticom Image Plus 2.0 software (Motic Group Co., Ltd. Xiamen, China): the body length (BL), which corresponded to the notochord length (from the tip of the snout to the tip of the notochord in pre-flexion larvae) or the standard length (from the tip of the snout to the base of the hypural bones in flexion and post-flexion larvae); the body height (BH) measured at the base of the pectoral



**Fig. 1.** Photographs of the larval clingfish (left, *Gobiesox marmoratus*; right, *Sicyases sanguineus*) and the sagitta otoliths. The arrow indicates the hatch mark (HM).

fin; and the body width (BW) measured between the pectoral fins. An estimation of larval volume was calculated by  $BL \times BH \times BW$  (in  $\text{mm}^3$ ), following Hovenkamp and Witte (1991). The larval measurements were not corrected for shrinkage and they were carried out less than a month from when larvae were collected.

The left and right sagittae otoliths were removed using insect needles from 269 individuals of larval *G. marmoratus* (2.67–8.62 mm SL) and 92 individuals of larval *S. sanguineus* (3.33–9.47 mm SL) (Fig. 1). The otoliths were embedded in epoxy resin on a glass slide. The daily age was determined by counting the number of otolith increments with a Motic BA310 light microscope (Motic Group Co., Ltd. Xiamen, China) at  $\times 1000$  magnification under oil immersion. The longest radius of a sagitta was measured three times and the average was used. The perimeters and areas of the otoliths were then measured once using the Moticam Image Plus 2.0 software.

Three independent counts were performed on the sagittae. The counts were performed after a prominent hatch mark (HM, Fig. 1). When the coefficient of variation ( $CV = \text{standard deviation}/\text{mean} \times 100$ ) of the increment counts among the three readings was  $< 5\%$ , the average of the three counts was calculated and utilised for the analysis. When the CV was  $> 5\%$ , the otolith reading was discarded. The daily periodicity of the growth increments for *G. marmoratus* and *S. sanguineus* have been recently validated (L. Mansur, G. Plaza, M. F. Landaeta, and F. P. Ojeda, unpubl. data). For the validation experiment, alizarin red-S was used as a chemical marker. This compound, which by means of epifluorescence-microscopy techniques reveals the number of rings deposited after fishes have been marked, was assimilated and incorporated into the calcareous structure of the otoliths (Campana 1990). The alizarin red-S was dissolved in two tanks ( $0.015 \text{ m}^3$ ) of seawater, at concentrations of  $150 \text{ mg L}^{-1}$ . The seawater remained heavily aerated to

maintain a pH near 7. Individuals were kept in the tanks with alizarin red-S for 24 h. Afterwards, individuals were placed in small aquaria ( $5 \text{ ind. aquarium}^{-1}$ ) with circulating, constantly aerated water and fed *ad libitum* for 6 days. After 6 days of acclimation, the specimens were subjected to 1 day of fasting and a second staining with alizarin red-S.

#### Data analysis

Least-square linear regression analyses were performed between the sagittae morphometry (radius and perimeter) and body length, and exponential models were fitted between the sagitta area and larval length. Univariate repeated-measures (RM) ANOVAs were performed to compare the daily increment widths of both species over time. Linear models between the species were compared with a one-way ANCOVA and multiple-slope test (Zar 1999). Additionally, linear regressions between the daily increment counts (age) and larval lengths were adjusted. In this case, the slope corresponded to the population growth rate, and the intercept corresponded to the estimated hatch size. To compare the population growth rate of both larval clingfishes, the slopes were compared with the multiple-slope test (Zar 1999).

The hatch-date composition of all measured larvae was subsequently estimated. Initially, the larval length-at-age was obtained using a linear model. The length–frequency distribution of the larvae was then converted to an age–frequency distribution using a length-at-age key, from which the hatching date compositions were back-calculated.

The back-calculated hatching dates were related to the lunar cycle. For each sampling date, the days since the new moon were counted (DNM), and thereby assigned DNM values from 0 to 29 for each date, in which 0 represented the new moon. The DNM values were converted to angles ( $^\circ$ ) by dividing by 29 (the length,

**Table 1.** Summary of the temporal variation in abundance and size range of larval *Gobiesox marmoratus* and *Sicyases sanguineus* collected in El Quisco Bay, central Chile, during the austral spring in 2010

| Date             | <i>Gobiesox marmoratus</i> |  |                                      |                     | <i>Sicyases sanguineus</i> |  |                                      |                     |
|------------------|----------------------------|--|--------------------------------------|---------------------|----------------------------|--|--------------------------------------|---------------------|
|                  | <i>n</i>                   | Abundance<br>(ind. 1000 m <sup>3</sup> ) | Range<br>(ind. 1000 m <sup>3</sup> ) | Larval size<br>(mm) | <i>n</i>                   | Abundance<br>(ind. 1000 m <sup>3</sup> ) | Range<br>(ind. 1000 m <sup>3</sup> ) | Larval size<br>(mm) |
| 2 September 2010 | 61                         | 28.9 ± 18.5                              | 5.9–73.9                             | 2.7–7.7             | 17                         | 11.8 ± 4.9                               | 5.3–20.1                             | 3.3–5.6             |
| 9 September 2010 | 137                        | 190.7 ± 324.4                            | 18.9–937.8                           | 3.1–8.6             | 48                         | 70.2 ± 124.7                             | 4.8–380.9                            | 4.0–9.5             |
| 4 October 2010   | 71                         | 26.8 ± 26.8                              | 4.1–110.2                            | 3.3–7.3             | 27                         | 20.0 ± 22.8                              | 3.5–77.2                             | 4.2–7.8             |

in days, of the lunar cycle) and then multiplying by 360°, so that the data could be analysed using circular statistics. To assess whether the hatching events showed lunar periodicity, the data were analysed using the Rao's spacing test (Batschelet 1981). The Rao's spacing test is more powerful and robust than many other circular goodness-of-fit-tests and is able to analyse bi- and multimodal distributions, whereas other tests, such as the Rayleigh test and Watson's  $U^2$ , cannot (Bergin 1991). The Rao's spacing test is robust even for small sample sizes but also shows a low frequency of Type I errors when analysing data that display no pattern. We also used the Rayleigh test for a departure from randomness. The null hypothesis that the hatching events would be equally or randomly spaced throughout the lunar cycle was tested for each dataset. The angular means and 95% confidence intervals were also calculated. Finally, comparisons between the lunar hatching distributions of both species were conducted using the non-parametric Mardia–Watson–Wheeler test (W) for equal distributions (Mardia 1972).

## Results

### Larval abundance and size distribution

The abundance of larval *G. marmoratus* (from 4 to 938 ind. per 1000 m<sup>3</sup>) and *S. sanguineus* varied among collections (from 4 to 381 ind. per 1000 m<sup>3</sup>, Table 1). Larvae smaller than 4 mm corresponded to 26.78% of *G. marmoratus* v. 1.42% for *S. sanguineus* (Fig. 2). The size ranges also varied between the species (Table 1); the collected larvae of *S. sanguineus* were significantly larger than those of *G. marmoratus* ( $U = 5993$ ,  $P < 0.001$ ). In relation to the larval volume, both larvae showed an exponential increase with size (Fig. 2); however, *G. marmoratus* larvae were more robust (larger volume) than *S. sanguineus* larvae, at identical body lengths (Fig. 2).

### Otolith morphometry

The sagittae otoliths showed significant relationships with the body length of both species (Fig. 3, Table 2), which suggests proportionality between the growth of the otolith and somatic growth of the fish. The radius and perimeter of the sagittae showed significant linear relationships with the BL of both species. The otolith area ( $A$ ) of *G. marmoratus* was related to the body length (BL), as represented by the model  $A = 365.11 \times \exp(0.409 \times \text{BL})$ . For larval *S. sanguineus*, the model was  $A = 474.01 \times \exp(0.272 \times \text{BL})$  (Fig. 3). Additionally, the otoliths of *G. marmoratus* grew approximately two times faster than did the otoliths of *S. sanguineus*, in both radius and

perimeter (one-way ANCOVA, radius,  $F = 24.48$ ,  $P < 0.001$ ; perimeter,  $F = 21.93$ ,  $P < 0.001$ ). Therefore, the sagittae otoliths of *G. marmoratus* were larger than those of *S. sanguineus*, at identical body lengths.

### Otolith microstructure and hatch marks

For larval *G. marmoratus*, the HM (Fig. 1) was evident in all analysed otoliths but varied widely from 12.60 to 36.00  $\mu\text{m}$  ( $22.42 \pm 5.03 \mu\text{m}$ ,  $\text{CV} = 22.41\%$ ). For *S. sanguineus*, the HM ranged between 8.80 and 23.30  $\mu\text{m}$  ( $14.15 \pm 2.50 \mu\text{m}$ ,  $\text{CV} = 17.68\%$ ). In both species, slight daily increments were observed between the primordium and hatch check (Fig. 1).

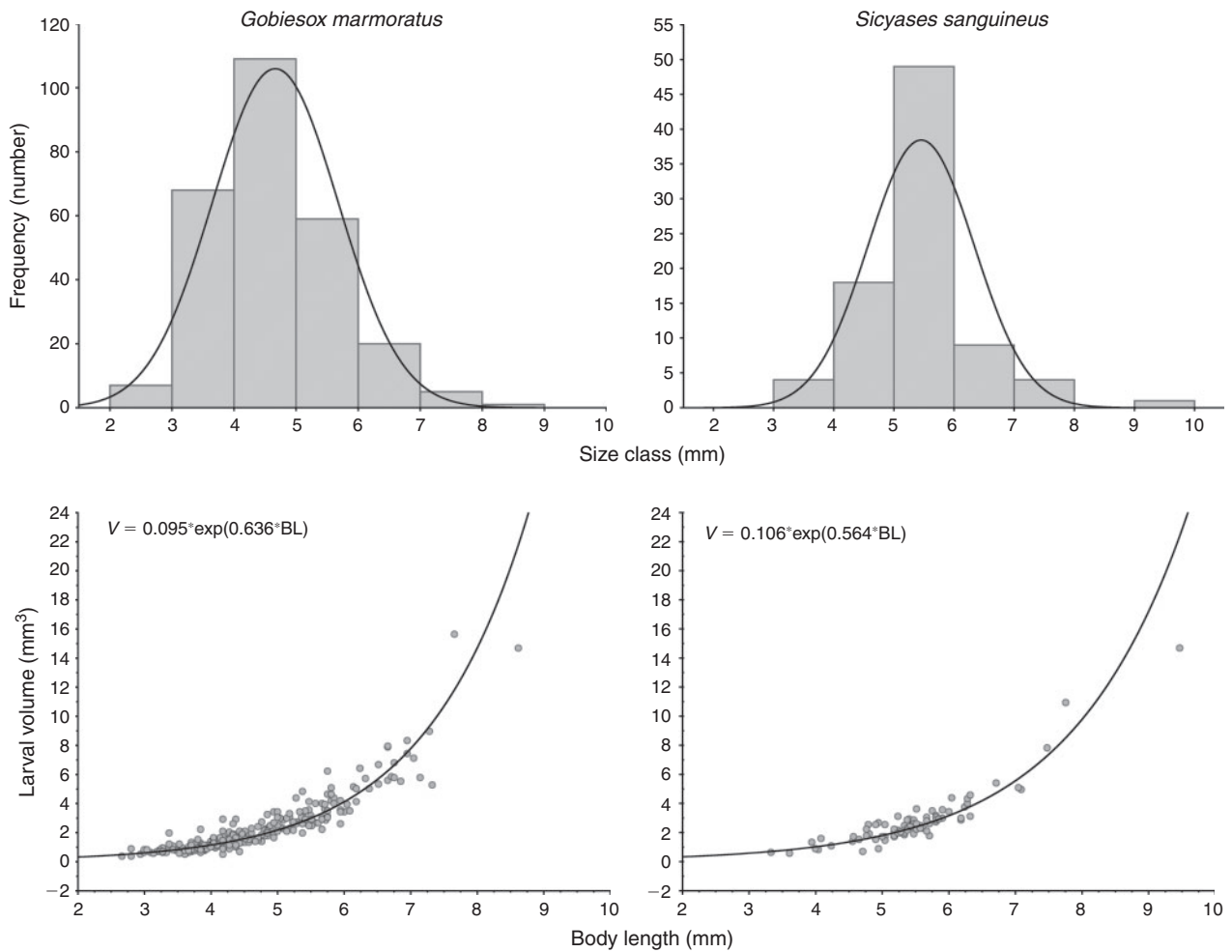
During the initial month of life, the daily increment widths ranged from 0.6 to 4.8  $\mu\text{m}$  ( $1.58 \pm 0.57 \mu\text{m}$ ,  $\text{CV} = 35.76\%$ ) in *G. marmoratus* and from 0.5 to 1.8  $\mu\text{m}$  ( $0.96 \pm 0.22 \mu\text{m}$ ,  $\text{CV} = 23.14\%$ ) in *S. sanguineus*. The growth trajectories (i.e. the daily increment widths) showed a larger daily variability for *G. marmoratus* than for *S. sanguineus* (Fig. 4, showing only older individuals of both species) and larger daily increment widths (univariate RM ANOVA; main effects for Species,  $F_{13,169} = 40.38$ ,  $P < 0.001$ ). Notably, there was not a significant increase in the widths over age in either *G. marmoratus* or in *S. sanguineus* (univariate RM ANOVA, Age  $\times$  Species interaction,  $F_{13,169} = 1.12$ ,  $P = 0.352$ ).

### Age and growth

For larval *G. marmoratus* and *S. sanguineus*, the within-individual age estimates using the left and right sagittae were identical (Wilcoxon matched-pairs test, *G. marmoratus*,  $P = 0.615$ ,  $n = 90$ ; *S. sanguineus*,  $P = 0.311$ ,  $n = 17$ ). Daily increment counts ranged from 1 to 24 in the right sagittae ( $n = 180$ ) of *G. marmoratus* and from 1 to 25 increments in the right sagittae of *S. sanguineus* ( $n = 49$ ). The linear larval-growth model for *G. marmoratus* was  $\beta = 0.24 \text{ mm day}^{-1}$ , with an estimated hatch size ( $\alpha$ ) of 3.45 mm (Table 3, Fig. 5). The estimated larval growth rate for *S. sanguineus* was  $0.14 \text{ mm day}^{-1}$ , with a hatch size of 3.88 mm. On the basis of linear regression models (Table 3), larval *G. marmoratus* hatched at a smaller size but grew faster than did larval *S. sanguineus* (one-way ANCOVA,  $F = 33.45$ ,  $P < 0.001$ ).

### Hatching patterns and lunar periodicity

For the study period, the estimates of the hatch days for *G. marmoratus* varied from Julian day 216 (4 August) to Day 276 (3 October) and from Julian day 213 (1 August) to Day 274 (1 October) for *S. sanguineus*. Two important hatching pulses



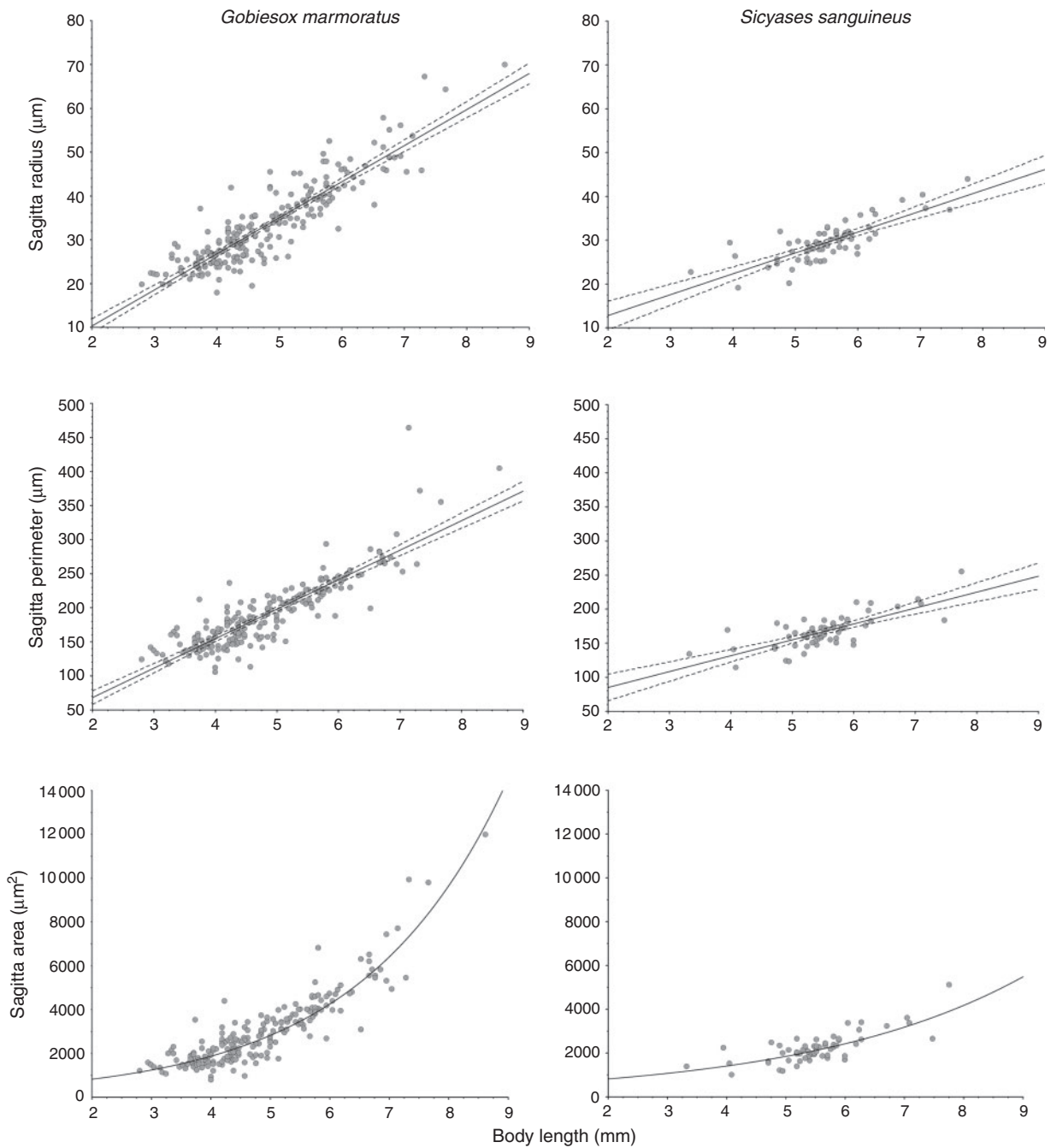
**Fig. 2.** Length-class frequencies and estimated larval volume (body length  $\times$  body height  $\times$  body width) of *Gobiesox marmoratus* and *Sicyases sanguineus* from central Chile collected during early spring in 2010.

were detected by the back-calculated hatch dates of *G. marmoratus*, and both occurred near the first-quarter moon (Fig. 6). However, the hatch-date frequencies of *S. sanguineus* were lower than those of *G. marmoratus* and were observed between the full moon and the first-quarter moon (Julian days 234–245) and between the third-quarter moon and first-quarter moon (Julian days 258 and 274) (Fig. 6). When all back-calculated hatching data were pooled, a large pulse of *G. marmoratus* hatching was observed during the first-quarter moon (i.e. a lunar periodicity, Fig. 7), and two pulses (i.e. semi-lunar periodicity) were evident for *S. sanguineus*, with one occurring in the first-quarter moon and the other near the full moon (Fig. 7). The angular mean (95% confidence interval) corresponded to Day 22 (21–23) of the lunar cycle for *G. marmoratus*; for *S. sanguineus*, the angular mean was approximately Day 16 (14–20) of the lunar cycle (Fig. 7). Rayleigh and Rao's spacing tests indicated that the hatching patterns of larval *G. marmoratus* and *S. sanguineus* were not uniform over the lunar cycle (*G. marmoratus*:  $r = 0.672$ ,  $P < 0.001$ , Rao's  $U = 319.6$ ,  $P < 0.001$ ; *S. sanguineus*:  $r = 0.245$ ,  $P < 0.001$ , Rao's  $U = 220.4$ ,  $P < 0.001$ ). Finally, significant differences were detected

among the lunar periodicities of hatching for both species ( $W = 15.85$ ,  $P = 0.00036$ ).

## Discussion

Larval clingfish have been scarcely observed in the plankton collections of central and southern Chile, particularly during the austral winter and spring, and to a lesser extent during the late summer (Balbontín and Bravo 1999; Bustos *et al.* 2008; Landaeta *et al.* 2008) unless those collections were performed close to shore (e.g. Hernández-Miranda *et al.* 2003; Vélez *et al.* 2005; the present study). This suggests that a spatial distribution of larvae is restricted to coastal areas, with there being a low probability of offshore advection and/or high net avoidance. A vertical distribution of larval clingfish restricted near the seafloor may also preclude collection, as has been detected in larval gobiesocids (*Lepadogaster candollei* and *L. lepadogaster*) from near-shore rocky substrates off Portugal (Beldade *et al.* 2006; Borges *et al.* 2007). In our study, larval clingfish were collected in relatively large abundance (from 6 to 900 larvae per 1000 m<sup>3</sup> for *G. marmoratus* and from 5 to 380 larvae



**Fig. 3.** Morphometric relationships of the sagittae otolith size (radius, perimeter and area) with the larval body sizes of clingfish from central Chile.

**Table 2.** Parameters of the linear models for the relationships between morphometry (radius and perimeter) of the sagittae and larval body length (BL) of clingfishes from central Chile  
 $\alpha$  = intercept,  $\beta$  = slope, s.e. = standard error

| Species                    | Comparison      | $\alpha$ | s.e.  | $\beta$ | s.e. | $R^2$ | $F$    | $P$    |
|----------------------------|-----------------|----------|-------|---------|------|-------|--------|--------|
| <i>Gobiesox marmoratus</i> | Radius v. BL    | -6.13    | 1.37  | 8.23    | 0.27 | 0.800 | 865.68 | <0.001 |
|                            | Perimeter v. BL | -18.42   | 8.28  | 43.27   | 1.69 | 0.759 | 654.09 | <0.001 |
| <i>Sicyases sanguineus</i> | Radius v. BL    | 3.26     | 2.54  | 4.76    | 0.45 | 0.632 | 108.44 | <0.001 |
|                            | Perimeter v. BL | 38.23    | 15.11 | 23.33   | 2.71 | 0.552 | 74.02  | <0.001 |

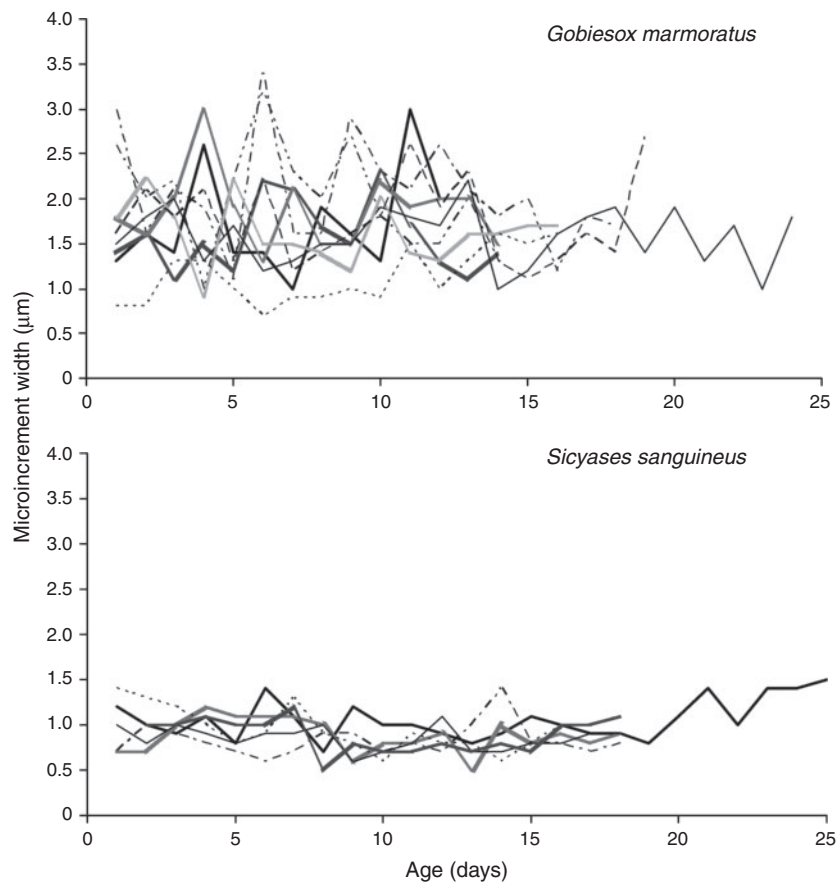


Fig. 4. Temporal variation of daily increment widths ( $\mu\text{m}$ ) during the larval development of clingfish (*Gobiesox marmoratus* and *Sicyases sanguineus*) from central Chile.

Table 3. Parameters of the linear models for the relationship between body length (mm) and number of daily increments (days) for larval clingfishes of central Chile

$\alpha$  = intercept (hatch size, mm),  $\beta$  = slope (population growth rate, mm day<sup>-1</sup>), s.e. = standard error

| Species                    | $\alpha$ | s.e. | $\beta$ | s.e. | $R^2$ | $F$    | $P$    |
|----------------------------|----------|------|---------|------|-------|--------|--------|
| <i>Gobiesox marmoratus</i> | 3.45     | 0.06 | 0.241   | 0.01 | 0.803 | 726.11 | <0.001 |
| <i>Sicyases sanguineus</i> | 3.88     | 0.12 | 0.144   | 0.01 | 0.838 | 207.07 | <0.001 |

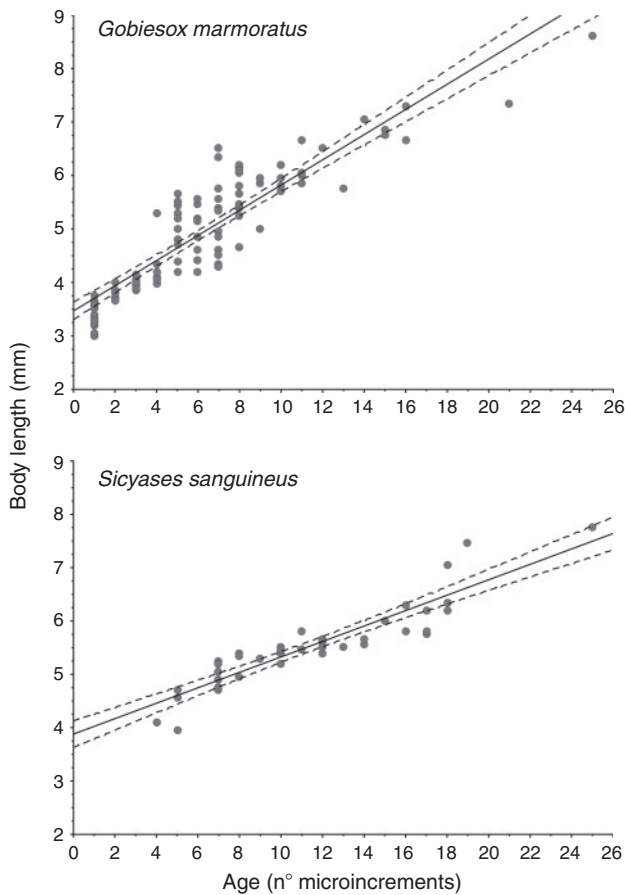
per 1000 m<sup>3</sup> for *S. sanguineus*), and *G. marmoratus* was always more abundant than *S. sanguineus* (Table 1). A similar trend in abundance was described for both species in a shallow embayment off of Peru (Vélez *et al.* 2005).

Additionally, larvae of *G. marmoratus* were more robust (heavier) than those of *S. sanguineus*. This robustness may have implications in the swimming abilities of each species. Clingfish larvae have slow swimming speeds (i.e. routine speed) and these are reduced in individuals approaching settlement size; this slow speed is related to behavioural changes associated with a benthic lifestyle and not to a decreased larval swimming ability. These individuals are completing metamorphosis and resemble newly settled fish, which show the presence of a ventral adhesive disk

(Faria and Gonçalves 2010). Therefore, it is expected that both species of Chilean clingfish have slow routine speeds and differ in swimming capabilities at a given size or age.

Larvae of *G. marmoratus* were larger at age at a specific age and grew at faster rates (0.24 v. 0.14 mm day<sup>-1</sup>) than did those of *S. sanguineus*, although the size of *G. marmoratus* at hatching was smaller than that of *S. sanguineus* (Pérez 1981; the present study). Faria and Gonçalves (2010) estimated growth rates of  $0.27 \pm 0.58$  mm day<sup>-1</sup> for larval *Lepadogaster purpurea* and  $0.19 \pm 1.04$  mm day<sup>-1</sup> for *L. lepadogaster*. Similarly, larval triplefin *Helcogrammoides chilensis*, collected in surveys identical to those of the larval gobiesocids analysed in the present study, grow at 0.19–0.21 mm day<sup>-1</sup> (Palacios-Fuentes *et al.* 2012). Faster larval growth should increase survivorship, and therefore result in higher levels of larval settlement or juvenile recruitment (Sponaugle 2010) and may have implications in the pelagic larval duration (PLD) of these species.

Lunar and semi-lunar hatching patterns are frequent in fishes with benthic eggs and other species showing viviparity (Plaza-Pastén *et al.* 2003). A hatching pattern related to the first-quarter moon (as was detected for both gobiesocids in the present study) is associated to neap tides, which implicate the reduced effects of tidal export from coastal waters to offshore (Robertson *et al.* 1990). In our study, both species significantly differed in the timing of hatching; *G. marmoratus* showed a lunar periodicity,

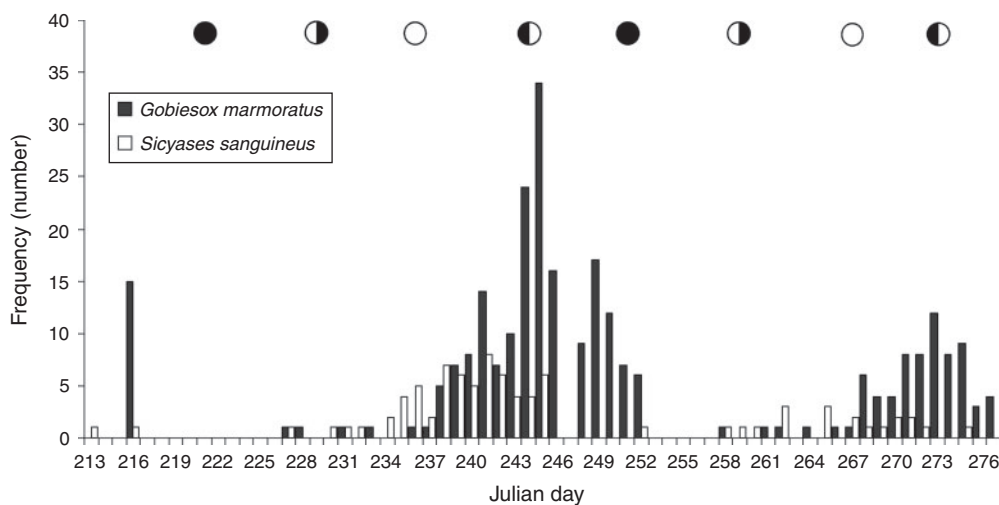


**Fig. 5.** Estimated growth models for both larval clingfish species from central Chile. Solid line indicates linear growth model. Dashed line indicates 95% confidence interval.

whereas *S. sanguineus* showed a semi-lunar periodicity in the hatching events (Fig. 7). If we consider that *S. sanguineus* larvae experience slower larval growth rates and hatch near a full moon, it is predicted that this species has enhanced their potential geographical dispersion more than has *G. marmoratus*. This enhanced dispersion is because one of the hatching pulses (during neap tide and first-quarter moon) may increase the near-shore retention potential, whereas the remaining pulse (during spring tide and full moon) may increase the probabilities of offshore advection and colonisation of distant niches.

In absence of selective mortality, the shapes of back-calculated hatch-date distributions and the observed larval-production distributions should be identical. However, if differences between the two distributions exist, such would suggest that the survival of larvae hatched on certain dates was enhanced relative to those hatched on alternate dates (Campana and Jones 1992). In the present case, most individuals of larval *G. marmoratus* collected were younger than 10 days old and, therefore, less biased by larval mortality. For *S. sanguineus*, larvae utilised in the back-calculated hatch dates were evenly distributed between 4 and 24 days old, and more bias may have occurred because the older survivors are proportionally more represented.

Both species deposit nest eggs along the rocky shore. *S. sanguineus* deposits nests of ~500 cm<sup>2</sup>, containing 25 000–29 000 spheroid eggs that measure 1.47–1.59 × 1.16–1.21 mm. The nests are deposited in exposed vertical rocky walls, and are guarded by one or two adults (Pérez 1981). However, *G. marmoratus* deposit egg nests of 300 cm<sup>2</sup> behind boulders, containing 19 000–23 000 spheroid eggs that measure 1.04–1.14 × 0.76–0.85 mm (Pérez 1981). The differences in the sizes of the nests and eggs are most likely related to differences in the maximum adult sizes between *G. marmoratus* and *S. sanguineus* (10- v. 30-cm total length, respectively). For both species, egg



**Fig. 6.** Back-calculated hatching patterns of clingfish from central Chile. The grey bar represents *Gobiesox marmoratus*, and the white bar represents *Sicyases sanguineus*.



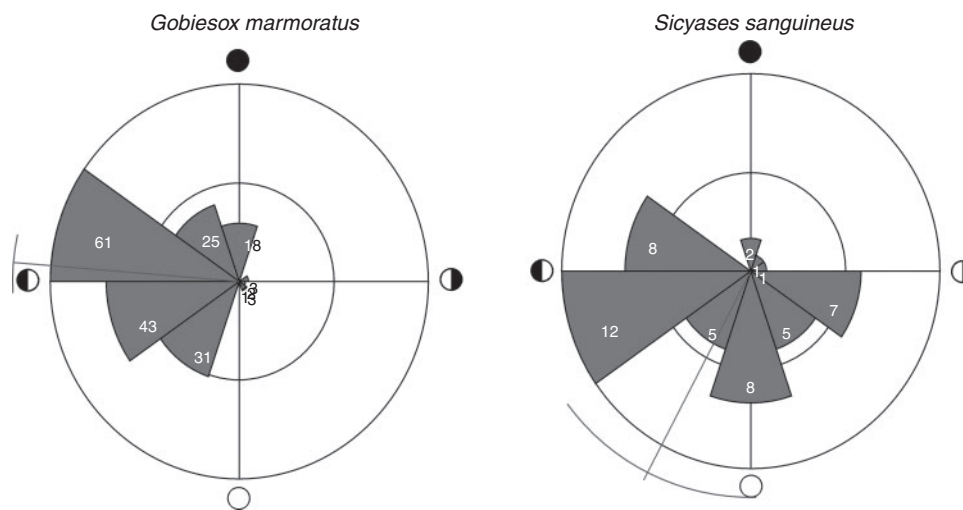


Fig. 7. Hatching patterns of clingfish from central Chile, based on the lunar cycle. Note that *Gobiesox marmoratus* shows a lunar periodicity and *Sicyases sanguineus* shows a semi-lunar periodicity.

development is relatively long and measures 20–21 days for *S. sanguineus* (cultured at 13–16°C) and 14–15 days for *G. marmoratus* (cultured at 16.5–17.7°C) (Pérez 1981). Egg development may explain the presence of embryonic micro-increment deposits in the otoliths before hatching, which are observed in all sagittae otoliths, and can also be observed in the embryos of *Oncorhynchus mykiss* (Mugiya 1987).

The timing of hatching for both species may vary largely according to our back-calculated data (several days around a specific moon phase) and large embryonic development. Both conditions may explain in part the large variability observed in the size of otolith HM, which is unusual in other fish species with pelagic eggs (Landaeta *et al.* 2012) or viviparity (Landaeta and Castro 2006). Another potential hypothesis to explain variability in size of otolith HM is related to temperature fluctuations in the near-shore area off central Chile. Temperature can vary 4°C between days and 3°C between day and night off Las Cruces, central Chile (Kaplan *et al.* 2003), just 15 km south to the area where larvae were collected. Also, there are important differences in water temperature between surface (i.e. in rocks below the levels of the lowest low tide) and 7-m depth (Kaplan *et al.* 2003), which is a bathymetric range where clingfishes are able to incubate their nests.

The daily increment counts observed in the sagittae of older larvae of both species in the present study suggest that the pelagic stage may be ~1 month. However, longer planktonic durations can be expected because the sizes of new settlers of both species in inter-tidal rocky pools observed in other studies are larger (~20-mm TL; Quijada and Cáceres 2000) than the upper range of larval size reported in the current study (~7 mm). Longer planktonic durations have also been reported in other species with benthic eggs and pelagic larvae from coastal Chile (e.g. the blenny *Scartichthys viridis*, 92–106 days, Hernández-Miranda *et al.* 2009; and the triplefin *H. chilensis*, 57 days, Palacios-Fuentes *et al.* 2012) and other cold-water temperate fishes (triplefin *Forsterygion lapillum*, ~50 days, Shima and Swearer 2009; and the *Merluccius hubbsi*, >60 days, Buratti and

Santos 2010). For other gobiesocids in the northern hemisphere, Beldade *et al.* (2007) reported a PLD of 11–18 days for *Apletodon dentatus*, *Lepadogaster lepadogaster*, *L. candollei* and *Opeatogenys gracilis*. These species inhabit warmer temperatures, which may reduce the pelagic larval duration and increase larval growth (Raventós and Macpherson 2001). More recently, Tojeira *et al.* (2012) calculated 33 days for larval development in *L. purpurea* cultured at 14.6°C. How can a reduced planktonic duration benefit the population? This tactic, together with a hatching pattern associated with a moon cycle that reduces offshore advection (during neap tides), may increase self-recruitment (Sponaugle *et al.* 2002).

In conclusion, the high abundance of larval clingfish in near-shore waters, the timing of decoupling among hatching events, and different growth rates may be tactics to increase self-recruitment in coastal waters and reduce interspecific competition among fish larvae with similar morphologies.

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