

**Abstract**—The long-snouted seahorse (*Hippocampus guttulatus*) (Cuvier, 1829), was used to validate the predictive accuracy of three progressively realistic models for estimating the realized annual fecundity of asynchronous, indeterminate, multiple spawners. Underwater surveys and catch data were used to estimate the duration of the reproductive season, female spawning frequency, male brooding frequency, and batch fecundity. The most realistic model, a generalization of the spawning fraction method, produced unbiased estimates of male brooding frequency (mean  $\pm$  standard deviation [SD]=4.2  $\pm$ 1.6 broods/year). Mean batch fecundity and realized annual fecundity were 213.9 ( $\pm$ 110.9) and 903.6 ( $\pm$ 522.4), respectively. However, females prepared significantly more clutches than the number of broods produced by males. Thus, methods that infer spawning frequency from patterns in female egg production may lead to significant overestimates of realized annual fecundity. The spawning fraction method is broadly applicable to many taxa that exhibit parental care and can be applied nondestructively to species for which conservation is a concern.

## Validation of a method for estimating realized annual fecundity in a multiple spawner, the long-snouted seahorse (*Hippocampus guttulatus*), using underwater visual census

**Janelle M. R. Curtis**

Email address: j.curtis@fisheries.ubc.ca  
Department of Biology  
McGill University  
1205 Dr. Penfield Avenue, Montréal  
Québec, Canada H3A 1B1

Present address: Project Seahorse  
Fisheries Centre  
University of British Columbia  
2202 Main Mall, Vancouver  
British Columbia, Canada V6T 1Z4

Reliable estimates of fecundity for multiple spawning fishes are difficult to obtain because of the logistic challenges associated with monitoring the reproductive activity of mature individuals over extended periods of time (Parrish et al., 1986). Fecundity estimates require knowing the number of young produced per spawning event (batch fecundity), as well as the number of batches of young produced per year (annual spawning frequency) (Hunter et al., 1986; Lowerre-Barbieri et al., 1996). Consequently, estimates of annual fecundity for multiple spawners often are derived from the sampling of ovaries to evaluate temporal patterns in the development and maturation of oocytes (Hunter and Leong, 1981; Murua and Saborido-Rey, 2003). However, the reliability of histological assessments is limited for estimating the annual fecundity of fishes with asynchronous oocyte development and indeterminate recruitment (e.g., anchovies, *Engraulis* spp.) (Brown-Peterson et al., 1988; Murua and Saborido-Rey, 2003). In these fishes, no one cohort of oocytes is dominant, and yolked oocytes forming new clutches are recruited from previtellogenic stages on a continual basis throughout the reproductive season (Wallace and Selman, 1981).

To obtain estimates of annual fecundity in asynchronous, indeterminate spawners, batch fecundity and spawning frequency are estimated by using different approaches. Batch fecundity can be obtained directly from the number of eggs released per spawning event or from the number of hydrated eggs present in the ovary immediately before spawning. Spawning frequency can be obtained indirectly by using a plot of the fraction of females spawning (indicated by the presence of hydrated eggs or new postovulatory follicles in the ovaries) over time (spawning fraction method, Hunter and Leong, 1981; Murua and Saborido-Rey, 2003).

Estimates of annual fecundity based on the number of eggs produced per year are reliable provided populations are sampled adequately and appropriately (DeMartini and Fountain, 1981; Hunter and Leong, 1981), and egg production is an unbiased proxy for the actual number of offspring produced. Histological methods for estimating annual fecundity may overestimate the actual number of young produced, or realized annual fecundity, if estimates do not correct for losses due to atresia, inability of females to secure mates, low fertilization efficiency,

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or clutch predation (Weddle and Burr, 1991; Cole and Sadovy, 1995). Such estimates of “potential” annual fecundity are usually difficult to validate because of the challenges of monitoring early life history stages (e.g., eggs, embryos, larvae) over time and space. In many species that provide parental care, however, both mating and brooding may be directly observed (e.g., Vincent and Giles, 2003). Such species provide an opportunity to validate indirect inferences based on egg production and to obtain more accurate estimates of the actual number of young produced (e.g., Cole and Sadovy, 1995). Seahorses are asynchronous, indeterminate multiple spawners that provide obligate paternal care in a sealed brood pouch (Boisseau, 1967; Wallace and Selman, 1981). Although numerous studies have investigated aspects of seahorse reproductive behavior (e.g., Vincent and Sadler, 1995; Perante et al., 2002), annual fecundity has been estimated for only one wild seahorse population (White’s seahorse, *Hippocampus whitei*, Vincent and Giles, 2003) by dividing the duration of the reproductive season by the brooding period (time required for a male to brood a clutch of eggs), and multiplying this estimate of spawning frequency by the average brood size (i.e., batch fecundity). Implicit in this method are three assumptions: 1) the time between release of young and remating is negligible, an assumption that was supported by observations of rapid remating in *H. whitei* (Vincent and Sadler, 1995), 2) individuals reproduce continuously from the beginning until the end of the reproductive season, and 3) there is no variation in brood size (a potentially unrealistic assumption, Vincent and Giles [2003]). If validated, this method could be used to estimate the realized annual fecundity of several *Hippocampus* spp. (IUCN<sup>1</sup>) with data that already exist (Foster and Vincent, 2004).

In this article, I used European long-snouted seahorses (*Hippocampus guttulatus*) (Cuvier, 1829), to validate three progressively realistic models for estimating spawning frequency and realized annual fecundity. The objectives of this study were 1) to estimate the batch fecundity and spawning frequency of *H. guttulatus*, 2) to identify correlates of fecundity, and 3) to evaluate the predictive accuracy of the three models. The simplest model is the calculation employed by Vincent and Giles (2003). The second model includes an estimate of interbrood interval. The most realistic model is a generalization of the “spawning fraction” method (Hunter and Leong, 1981) that could be applied nondestructively to estimate realized annual fecundity in species of conservation concern. The study objectives were addressed by using fishery-independent sampling and underwater visual census in a locally abundant and unexploited population of *H. guttulatus*.

<sup>1</sup> IUCN (World Conservation Union). 2006. 2006 IUCN Red List of Threatened Species. IUCN Species Survival Commission, 219c Huntingdon Road, Cambridge CB3 0DL, United Kingdom. Website: <http://www.redlist.org> (accessed 31 May 2006).

## Materials And Methods

### Species description

The life history and ecology of seahorses has been reviewed by Foster and Vincent (2004). *Hippocampus guttulatus* inhabits seagrass- and macroalgae-dominated communities in the Mediterranean Sea and the north-eastern Atlantic Ocean (Lourie et al., 1999). Individuals begin reproducing at approximately 1 year of age and live 4 to 5.5 years (Boisseau, 1967; Curtis and Vincent, 2006). Adults range in size from 110 to 210 mm standard length (sum of head, trunk, and tail lengths, Lourie et al., 1999) and from 2.4 to 22.5 g wet mass (Curtis and Vincent, 2006). Male and female seahorses mate monogamously within reproductive cycles: the female deposits an entire clutch of eggs into the male’s brood pouch (Jones et al., 1998). Brooding male *H. guttulatus* (i.e., with full pouches) have been captured from March to October (Boisseau, 1967; Reina-Hervás, 1989) and in January (Lo Bianco, 1888). The ovarian structure of *H. guttulatus* indicates that females produce multiple clutches per year (Boisseau, 1967).

### Sampling

This study was carried out in the Ria Formosa lagoon in southern Portugal (36°59’N, 7°51’W). The Ria Formosa is a shallow, productive coastal lagoon characterized by high water turnover rates, seagrass beds, sand flats, salt marshes, and a network of channels and tidal creeks (Machás and Santos, 1999; Curtis and Vincent, 2005). Data employed in this study were derived from fisheries-independent samples of fish community structure (Erzini et al.<sup>2</sup>) and from underwater visual censuses of tagged *H. guttulatus* on a small focal study site (Curtis and Vincent, 2006). Seahorses captured during fisheries-independent sampling were collected monthly from September 2000 to July 2002 (except January 2002) at 53 stations throughout the western part of the Ria Formosa lagoon by using small beach seines, push nets, or beam trawls (Erzini et al.<sup>2</sup>), and then frozen. Data from all the captured seahorses were opportunistically recorded and included the standard length, life history stage, sex, reproductive state, wet mass, and brood size of males with full pouches (details provided in Curtis, 2004; Curtis and Vincent, 2006). Brood sizes were obtained by dissecting the full pouches of captured males and counting all of the developing embryos. Broods with embryos captured during the earliest stages of development (<stage 10, *sensu* Boisseau, 1967) were

<sup>2</sup> Erzini, K., L. Bentes, R. Coelho, C. Correia, P. G. Lino, P. Monteiro, J. Ribeiro, and J. M. S. Gonçalves. 2002. Recruitment of sea breams (Sparidae) and other commercially important species in the Algarve (southern Portugal). Final Report, 178 p. Commission of the European Communities DG XIV/C/1. Directorate-General for Fisheries and Maritime Affairs, Unit for Information and Communication, European Commission, B-1049, Brussels, Belgium.

excluded from the analyses because eggs and small embryos were in poor condition due to freezing and were therefore difficult to count. For the same reason, it was not possible to estimate clutch sizes by examining the ovaries of captured adult females. Data were recorded from a total of 1264 adult males and 1211 adult females captured with the fishing gears (Erzini et al.<sup>2</sup>).

Underwater visual censuses of tagged *H. guttulatus* were also carried out on a 10 m × 10 m focal study grid established in June 2001. The grid was located 5 m from the intertidal zone next to a permanent pier located within the Ria Formosa Natural Park at a depth of 1.3 to 6.1 m (additional details provided in Curtis, 2006; Curtis and Vincent, 2006). Tagged seahorses exhibited high site fidelity to small home ranges averaging 20 m<sup>2</sup> during multiple years (Curtis and Vincent, 2006). The juveniles released by eight tagged males on the grid were also counted and used to estimate brood size as in Vincent and Giles (2003). The eight brooding males were placed in cages (0.3 m high × 0.45 m wide, 0.5 mm mesh) *in situ* for a maximum of 48 h each between 18 July and 16 August 2002 (Curtis and Vincent, 2006). The mesh allowed water and small zooplankton to flow through the cages, while retaining newborn juveniles. After juveniles were born, the cages were slowly brought to the surface and emptied into large tanks filled with seawater where the total number of juveniles was counted. Males and their juveniles were released at their original capture site within 12 hours of birth.

### Three models for estimating annual fecundity

The assumptions of rapid remating and continuous reproduction during the reproductive season were examined by using three progressively realistic models to estimate realized annual fecundity: the continuous reproduction (CR) model, the intermittent reproduction (IR) model, and the intermittent and seasonal reproduction (ISR) model.

#### Continuous reproduction (CR) model

In the simplest model, individuals were assumed to reproduce continuously from the beginning to the end of the reproductive season, from March to October (Boisseau, 1967; Reina-Hervás, 1989). Annual fecundity,  $f_{b, yr}$ , was estimated by using mean estimates of the brooding period,  $t_b$ , and brood size,  $f_b$ :

$$f_{b, yr} = f_b \times t_s / t_b, \quad (1)$$

where  $f_{b, yr}$  = the total number of young produced per male per year; and

$t_s$  = the duration of the reproductive season.

In the CR model,  $f_b$  and  $t_b$  were assumed to be constant throughout  $t_s$ , and the interbrood interval,  $t_{ib}$ , was 0. The brooding period,  $t_b$ , was estimated by monitoring changes in the reproductive state of tagged adult males

on the grid using underwater visual census. In 2001 and 2002, 553 *H. guttulatus* (264 males, 254 females, 35 juveniles) were individually tagged with visible implanted fluorescent elastomer (VIE tags, Northwest Marine Technologies, Inc., Shaw Island, WA) (Curtis, 2006; Curtis and Vincent, 2006). A total of 163 underwater visual censuses (2–5 person-hours/dive) were carried out with SCUBA on the grid during two census periods: from 17 July to 26 October 2001 and from 23 May to 12 September 2002. On average, one underwater visual census was carried out every 1 to 2 days during each of these census periods. During underwater visual censuses, observers swam ~1 m above the substrate and searched for tagged seahorses. The date, tag, life history stage, sex, size (trunk length, converted to standard length by using equations developed for *H. guttulatus*, Curtis and Vincent 2005, 2006), and reproductive state of all tagged seahorses were noted. The reproductive state of adult males (full or empty brood pouch) was determined by visual (and in ambiguous cases, manual) inspection.

Changes in reproductive state were plotted as a function of census date for each tagged male. Because of high seahorse density on the grid (1.5/m<sup>2</sup>; Curtis and Vincent, 2006), it was not feasible to verify the status of all individuals during each dive; therefore,  $t_b$  was estimated by recording the minimum and maximum possible durations of each brooding period, as graphically depicted in Figure 1. Unmated male *H. guttulatus* occasionally mimic males that are close to releasing juveniles by temporarily and completely filling their pouches with water during reproductive displays to females (J. Curtis, personal observ.). Reproductive interactions between male and female *H. guttulatus* generally occurred within two hours of sunrise and lasted on average 4.8 min ± 3.3 SD (standard deviation) (J. Curtis, unpubl. data). Therefore putative brooding periods were included in the analysis only when a male was observed ≥ 3 times with a full pouch during a period bracketed by observations of an empty pouch. The minimum and maximum possible brooding periods were averaged within and among all males.

#### Intermittent reproduction (IR) model

This model expanded the continuous reproduction model by incorporating a mean estimate of the interbrood interval,  $t_{ib}$ , which was assumed to be constant throughout the reproductive season:

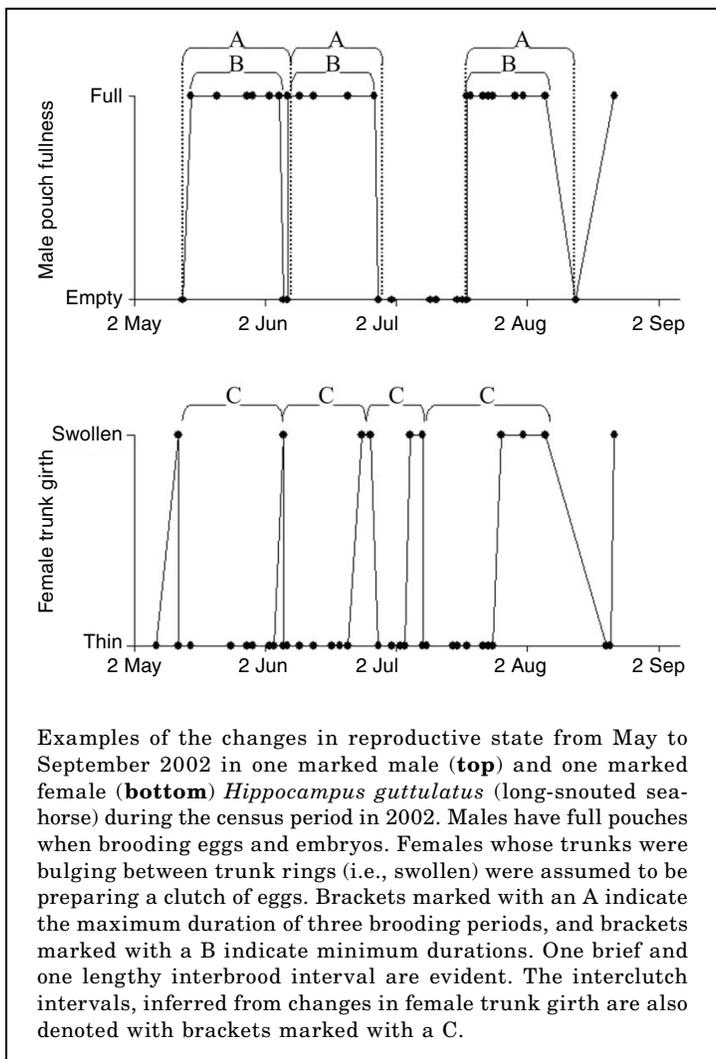
$$f_{b, yr} = f_b \cdot t_s / (t_b + t_{ib}), \quad (2)$$

where  $t_{ib}$  = the time elapsed between release of young and deposition of a new clutch of eggs into the male's brood pouch.

The interbrood interval was estimated by using a similar approach to that employed to estimate the brooding period (Fig. 1): the maximum and minimum possible durations of each interbrood interval were recorded and

averaged within and among tagged males observed on the grid in 2001 and 2002.

For comparison, the annual frequency of clutches prepared by tagged females was also estimated by monitoring the time required to prepare eggs,  $t_c$ , and the interval between inferred clutches,  $t_{ic}$  (Fig. 1). Female trunk girth was used as an indicator of reproductive status (Vincent and Sadler, 1995; Perante et al., 2002). Females whose trunks were bulging between trunk rings (occasionally with visible eggs) were assumed to be preparing a clutch of eggs for mating. Within monogamous pairs of *H. comes* (Perante et al., 2002) and *H. whitei* (Vincent and Sadler, 1995), changes in female trunk girth were significantly correlated with changes in the reproductive state of their male partner. Therefore trunk girth was assumed to be a reliable proxy for clutch preparation in *H. guttulatus*. Because female sea pony (*Hippocampus fuscus*), prepared and released their eggs within approximately three days (Vincent, 1990),  $t_{ic}$  was estimated only during periods when female *H. guttulatus* were observed on average at least once every two to three days.



### Intermittent and seasonal reproduction (ISR) model

This model is more realistic than the two previous models because information about seasonal trends in reproductive activity is incorporated, rather than the assumption that there is constant reproduction throughout the reproductive season. This model was adapted from the spawning fraction method used to estimate the average number of spawnings per year among female northern anchovy (Hunter and Leong, 1981). A Gaussian curve was fitted to the fraction of mature males with full pouches in the catch data plotted against sampling month by using nonlinear regression, as in Curtis and Vincent (2006). The area beneath this curve corresponds to an estimate of the total number of days the average adult male brooded embryos per year,  $t_{b,yr}$ . The mean number of broods produced per male each year (i.e., annual spawning frequency),  $s_{b,yr}$ , was estimated as  $t_{b,yr}/t_b$ . Annual fecundity was then calculated as  $f_b s_{b,yr}$ . Similarly, the ISR model was applied to the proportion of adult females preparing eggs, where the number of clutches produced by females each year,  $s_{c,yr}$ , was estimated as the total time spent preparing clutches of eggs,  $t_{c,yr}$ , divided by the time required to prepare a single clutch,  $t_c$ . In order to characterize among-population differences in *H. guttulatus* spawning frequency, this method was also applied to male catch data reported from the Arcachon Basin, France (Boisseau, 1967).

### Model validation

An independent estimate of the brooding period (21 days; Boisseau, 1967) was used to evaluate the predictive accuracy of the CR and ISR models. The CR model was used to predict the expected number of broods produced by males,  $s_{b,cen}$ , during the census periods from 18 July to 26 October (2001, 112 days) and 23 May to 12 September (2002, 108 days). Similarly, the curve fitted to the male catch data using the ISR model was used to calculate the predicted number of broods produced by males during these two census periods. Because the only estimate of the time females take to prepare eggs,  $t_c$  (used to estimate the number of clutches produced by females in the ISR model) was based on the underwater visual census data, the ISR model could not be used to predict the total expected number of clutches produced by females during the census periods. Therefore, the ISR model was used to predict the total number of days females spent preparing eggs during the census periods,  $t_{c,cen}$ . Expected values of  $s_{b,cen}$  and  $t_{c,cen}$  based on the CR and ISR models were compared to the mean values of  $s_{b,cen}$  and  $t_{c,cen}$  directly observed on the grid during the corresponding census periods. The IR model could not be validated because there was no independent estimate of interbrood interval,  $t_{ib}$ , for *H. guttulatus*.

### Correlates of fecundity

The influences of body size, water temperature, season, and lunar phase on batch fecundity and reproductive activity (i.e., the proportion of spawning males and females) were investigated. Daily and mean monthly water temperatures in the Ria Formosa were recorded with an Onset HOBO Temperature logger (Onset Computer, Bourne, MA) placed 0.3 m above the substrate on the grid from June 2001 to September 2002. All means are reported with one standard deviation or 95% confidence intervals (95% CI). Statistical procedures were carried out with SPSS 10.0.7 (SPSS Inc., Chicago, IL).

### Results

#### Continuous reproduction (CR) model

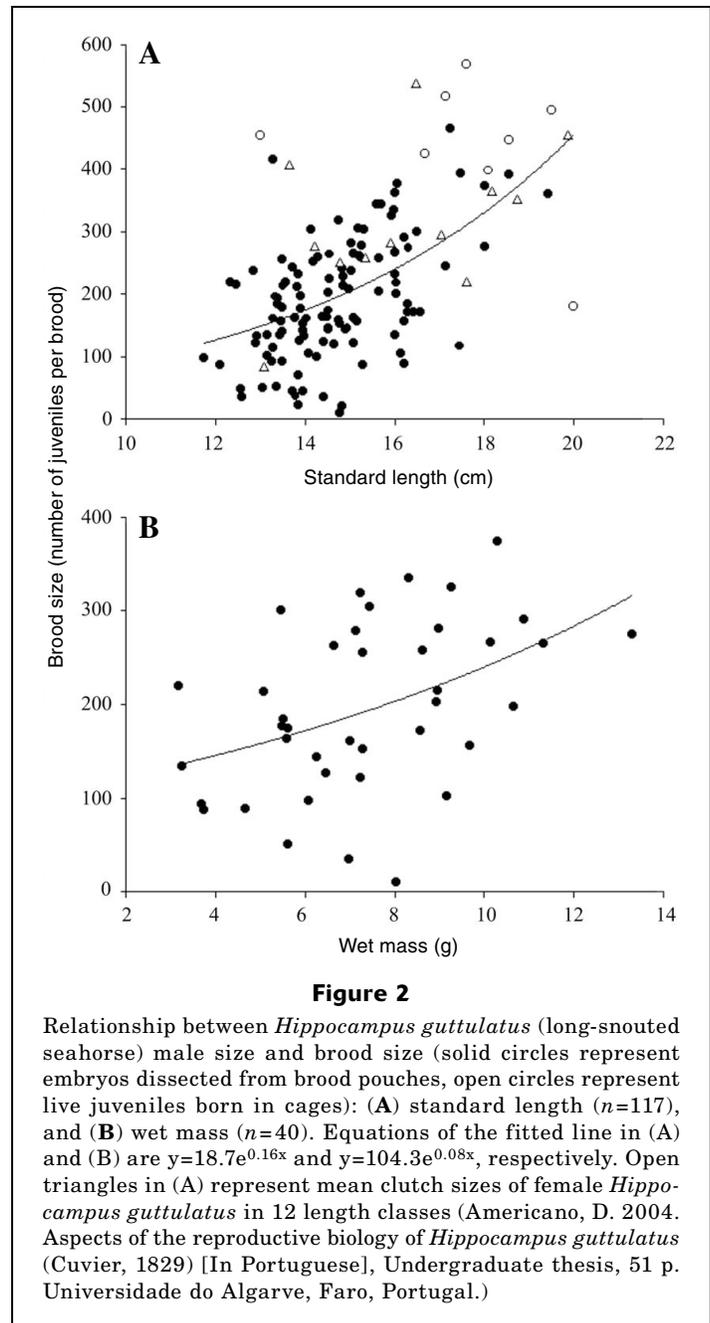
Nonlinear regression indicated that the size of male *H. guttulatus* was a significant predictor of brood size,  $f_b$ , which varied from 10 to 567 embryos or juveniles, with a mean of  $213.9 \pm 110.9$  ( $n=117$ , caged and dissected males pooled). Both standard length ( $r^2=0.29$ ,  $P<0.0001$ ,  $n=117$ ) and wet mass ( $r^2=0.20$ ,  $P<0.003$ ,  $n=39$ ) significantly predicted  $f_b$  (Fig. 2). Brood size was not correlated with mean monthly temperature ( $r^2=0.00$ ,  $P=0.90$ ,  $n=117$ ) but was weakly correlated with Julian day in 2001 ( $r^2=0.06$ ,  $P=0.015$ ,  $n=106$ ).

A total of 4550 records were collected from the 553 individually tagged *H. guttulatus* in 2001 and 2002 for an average of 8.2 records per individual (maximum 87). On the grid, 94% of resighted, mature males mated at least once within a census period. The mean brooding period,  $t_b$ , was  $21.4 (\pm 5.5)$  days ( $n=80$  intervals from 73 males, median=20.0 days). Temperature was not a significant predictor of  $t_b$  ( $r^2=0.004$ ,  $F_{[1,55]}=0.22$ ,  $P>0.05$ ), despite reports of an inverse relationship between temperature and brooding period in other seahorse species (Vincent and Sadler, 1995; Foster and Vincent, 2004).

The CR model produced the highest estimates of annual spawning frequency,  $s_{b,yr}$ , and realized annual fecundity,  $f_{b,yr}$ . Assuming continuous reproduction from March to October (245 days), male *H. guttulatus* was predicted to produce 11.5 broods annually. Mean annual fecundity was estimated as approximately 2450 young per year (Table 1).

#### Intermittent reproduction (IR) model

The interbrood interval,  $t_{ib}$ , ranged from 0 to 61 days and had a mean of  $12.9 (\pm 8.2)$  days ( $n=98$  intervals from 58 males; median=10.5 days) (Table 1). Size did not influence the number of broods produced by males during the census periods (Kruskal-Wallis test,



$\chi^2=3.0$ ,  $n=73$ ,  $df=5$ ,  $P=0.7$ ) and temperature was not a significant predictor of  $t_{ib}$  ( $r^2=0.001$ ,  $F_{[1,97]}=0.07$ ,  $P=0.79$ ). Incorporating  $t_{ib}$  into the IR model led to substantially lower estimates of spawning frequency and annual fecundity than predicted by the CR model:  $7.14 (\pm 4.89)$  broods per year and  $1527 (\pm 1309)$  young per year, respectively (Table 1).

All resighted, mature females prepared at least one clutch of eggs within a census period on the grid. The mean number of days females had hydrated eggs,  $t_c$ , was  $2.58 (\pm 2.66)$  days ( $n=72$  inferred clutches), indicating that females required at least 2.5 days to prepare

**Table 1**

Parameter values ( $\pm$  standard deviation) and model predictions from three progressively realistic models for the Ria Formosa population of *Hippocampus guttulatus* (long-snouted seahorse). Estimates are also given for the Arcachon Basin population by using the intermittent and seasonal reproduction model.

Model	Continuous reproduction (CR)	Intermittent reproduction (IR)	Intermittent and seasonal reproduction (ISR)	Intermittent and seasonal reproduction (ISR)
Population	Ria Formosa	Ria Formosa	Ria Formosa	Arcachon Basin
<b>Parameters</b>				
Reproductive season $t_s$ (days)	245 <sup>1</sup>	245 <sup>1</sup>	250 <sup>2</sup>	102 <sup>2</sup>
Brooding period $t_b$ (days)	21.4 ( $\pm 5.6$ )	21.4 ( $\pm 5.6$ )	21.4 ( $\pm 5.6$ )	21 <sup>3</sup>
Interbrood interval $t_{ib}$ (days)	0	12.9 ( $\pm 8.2$ )		
Time spent brooding $t_{b,yr}$ (days)			90.4	20.7
Brood size (batch fecundity) $f_b$	213.9 ( $\pm 110.9$ )	213.9 ( $\pm 110.9$ )	213.9 ( $\pm 110.9$ )	232 ( $\pm 82.8$ ) <sup>3</sup>
<b>Predictions</b>				
Spawning frequency $s_{b,yr}$	11.5 ( $\pm 2.9$ )	7.14 ( $\pm 4.89$ )	4.22 ( $\pm 1.09$ )	0.98
Annual fecundity $f_{b,yr}$	2449.2 ( $\pm 1415.9$ )	1527.2 ( $\pm 1309.8$ )	903.6 ( $\pm 522.4$ )	227.4 ( $\pm 81.6$ )

<sup>1</sup> Assuming 8 months from March through October (Reina-Hervás, 1989).

<sup>2</sup> Period when  $\geq 1\%$  of mature males were predicted to have full pouches.

<sup>3</sup> Values reported in Boisseau (1967).

eggs for mating. Although there was no significant difference between the brooding period,  $t_b$ , of males and female interclutch interval,  $t_{ic}$  (21.06  $\pm$  6.6 days,  $n=102$  intervals from 26 females; median=20 days) ( $t$ -test,  $df=180$ ,  $t=-0.36$ ,  $P=0.72$ ), females produced significantly more clutches than males brooded during both underwater visual census periods (2001:  $df=37$ ,  $t=3.32$ ,  $P=0.002$ ; 2002:  $df=62$ ,  $t=7.16$ ,  $P<0.0001$ , Table 2). Assuming that the duration of the reproductive season is the same for both sexes and that the IR model is correct, the estimate of the interclutch interval indicates that the average female prepared 11.63 ( $\pm 3.65$ ) clutches per year. Temperature was not a significant predictor of  $t_{ic}$  ( $r^2=0.02$ ,  $F_{[1,101]}=1.66$ ,  $P=0.2$ ).

#### Intermittent and seasonal reproduction (ISR) model

The reproductive activity of males varied significantly within years ( $r^2=0.78$ ,  $F_{[2,19]}=39.28$ ,  $P<0.0001$ , Fig. 3A). *Hippocampus guttulatus* males reproduced during most of the year and peaked in reproductive activity from June through August. No reproductively active males were captured from December 2000 to March 2001, or in February 2002. Male reproductive activity was also significantly and positively correlated with water temperature ( $r^2=0.55$ ,  $F_{[1,12]}=13.32$ ,  $P=0.004$ ), which varied seasonally from 10–28°C (mean annual temperature=18.2°C). There was no effect of lunar phase on male reproductive activity ( $r^2=0.01$ ,  $F_{[2,50]}=0.26$ ,  $P=0.78$ ), as inferred by Boisseau (1967). The fraction of mature females that were preparing eggs also varied seasonally ( $r^2=0.21$ ,  $F_{[2,17]}=3.59$ ,  $P=0.05$ , Fig. 3B). Temperature was weakly correlated with female reproductive

activity ( $r^2=0.30$ ,  $F_{[1,12]}=4.80$ ,  $P=0.05$ ), but lunar phase was not ( $r^2=0.04$ ,  $F_{[2,50]}=1.15$ ,  $P=0.33$ ).

Incorporation of seasonal variation in reproductive activity into the ISR model resulted in estimates of  $s_{b,yr}$  and  $f_{b,yr}$  that were 63.1% smaller than those predicted by the CR model. The ISR model predicted that the total time an average male *H. guttulatus* spent brooding embryos per year in the Ria Formosa was 90.4 days, giving an estimate of male annual spawning frequency,  $s_{b,yr}$ , of 4.22 broods per year (Table 1). Similarly, the total time females spent preparing eggs per year was 13.51 days, giving an estimate of female annual spawning frequency,  $s_{c,yr}$ , equal to 5.23 clutches per year. These estimates of annual spawning frequency were at least four times greater than estimates from the Arcachon Basin population. During the reproductive season, which lasted 3.5 months (Fig. 4), the average male had a full pouch for 20.7 days, indicating that, on average, males in the Arcachon Basin population bred only once a year (Table 1).

#### Model validation

The number of broods predicted by the CR model for the 2001 and 2002 census periods,  $s_{b,cen}$ , was significantly greater than observed directly with underwater visual census (Table 2), indicating that this model produces upwardly biased estimates of spawning frequency for *H. guttulatus*. By contrast, the more realistic ISR model produced expected values of  $s_{b,cen}$  and the total number of days female spent with hydrated eggs,  $t_{c,cen}$ , which were within 95% confidence intervals for the observed mean values on the grid in both 2001 and 2002. This

latter inference is supported by close correspondence between observed male and female reproductive activity observed over time on the grid and the values predicted by the ISR model (Fig. 3).

## Discussion

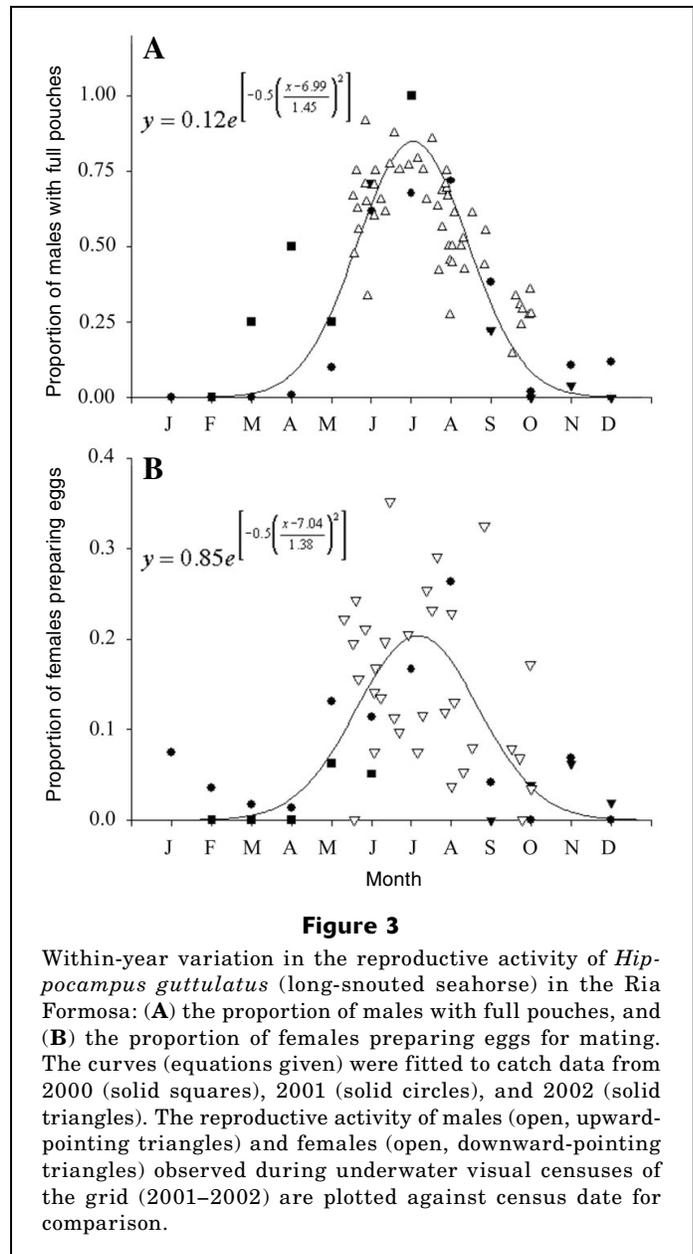
This article presents the first estimates of realized annual fecundity in a population of wild European long-snouted seahorses (*H. guttulatus*) and validates a potentially nondestructive application of the spawning fraction method (Hunter and Leong, 1981) for estimating spawning frequency. Results indicate that estimates of spawning frequency and annual fecundity based on egg production alone may lead to significant overestimates of realized annual fecundity and thus underscore the importance of testing assumptions when using proxies for estimating fecundity in multiple spawning fishes.

## Batch fecundity

A significant relationship between male size and brood size of *H. guttulatus* means that size-specific realized annual fecundities can be calculated and converted to age-specific fecundities (e.g., Curtis, 2004) by using a length-at-age model developed for *H. guttulatus* (Curtis and Vincent, 2006). Among fishes, fecundity is strongly size-dependent (Davis and West, 1993; Lowerre-Barbieri et al., 1996). Female size is typically used to predict clutch size in fishes (Bagenal, 1978), including syngnathids (Teixeira and Musick, 2001; Vincent and Giles, 2003), but male dimensions can be also used to predict brood size in fishes that provide paternal care, such as syngnathids (Strawn, 1958; Teixeira and Musick, 2001) and mouthbrooding cardinalfishes (Okuda et al., 1998; Kolm, 2002). Because both the volume of the female's abdominal cavity and volume of the male's sealed brood pouch potentially limit the number or size (or both) of embryos that can be successfully produced by seahorses (Boisseau, 1967), correlations between the dimensions of both parents and brood size likely reflect mutual mate selection for size (Vincent and Sadler, 1995; Teixeira and Musick, 2001; Vincent and Giles, 2003). A positive correlation between the standard lengths of *H. guttulatus* males and females engaged in courtship behavior on the grid (J. Curtis, unpubl. data), indicates that mating is size-assortative in this population.

## Annual spawning frequency

The underwater visual census data indicate that on average, female *H. guttulatus* prepared significantly more clutches of eggs (1.2–1.7 times as many) than males brooded in the Ria Formosa in 2001 and 2002 (Table 2). This means that estimates of spawning frequency and annual fecundity based on female egg production (e.g.,



histological assessments) may significantly overestimate the actual number of young produced. Brood production by males was probably not limited by the availability of mature females because sex ratios were slightly biased in favor of females (Curtis and Vincent, 2006), and the interclutch intervals of females were equal to the brooding periods of males. Significantly longer interbrood intervals for the population of male *H. guttulatus* in the present study may have derived in part from depredation by other fishes during egg transfer to male brood pouches. This depredation occurred during one of three matings witnessed on the grid. Approximately 1% of male *H. guttulatus* in the Ria Formosa had holes in their pouch. Such injuries indicate that other predators

**Table 2**

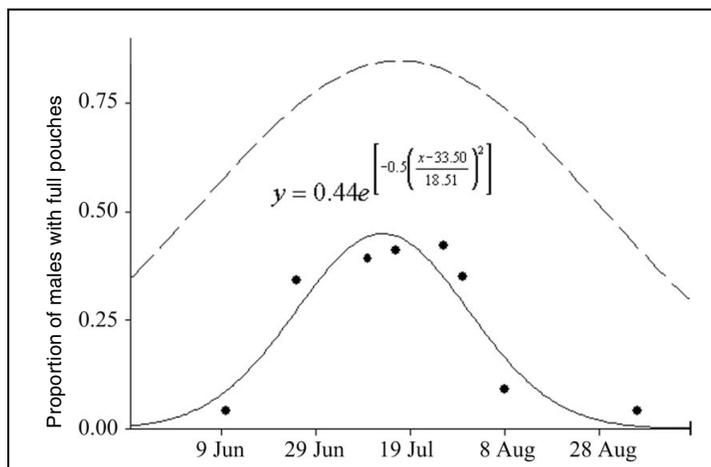
Predicted and observed number of broods (males) and clutches (females) produced during underwater visual census periods in 2001 and 2002. Expected values were calculated by estimating the area beneath fitted curves in Figure 3 that corresponded to census periods in 2001 and 2002. Observed values (mean  $\pm$  standard deviation) were taken from  $n$  males and females that were tagged and monitored throughout the entire census period. \* = greater than upper 95% confidence limit of mean observed value.

	2001		2002	
	17 Jul–26 Oct	$n$	23 May–12 Sept	$n$
<b>Males</b>				
Observed number of broods (95% confidence interval)	2.07 $\pm$ 1.10 (0–4.22)	28	2.72 $\pm$ 0.86 (1.03–4.40)	45
Continuous reproduction (CR) model Predicted number of broods	5.33*		5.14*	
Intermittent and seasonal reproduction (ISR) model Predicted days with full pouch	56.65		71.59	
Predicted number of broods	2.69		3.40	
<b>Females</b>				
Observed number of clutches (95% confidence interval)	3.40 $\pm$ 1.07 (1.30–5.49)	10	4.66 $\pm$ 1.23 (2.24–7.07)	18
Observed days with prepared eggs (95% confidence interval)	8.51 $\pm$ 3.37 (1.88–15.11)		11.58 $\pm$ 5.13 (1.52–21.65)	
Intermittent and seasonal reproduction (ISR) model Predicted days with prepared eggs	8.14		10.46	

may also depredate eggs or developing embryos directly from the pouch (Curtis, 2004). A wounded wild *H. whitei* male had a hole in his pouch that precluded mating for nearly three reproductive cycles (~60 days, Vincent and Sadler, 1995). The degree to which predation affects reproductive success in *H. guttulatus* is unknown, but

reproductive success in other fishes that provide male parental care is strongly affected by interspecific predation (e.g., Cole and Sadovy, 1995).

A fourfold difference in estimated spawning frequency between the Arcachon Basin (one brood per year) and the Ria Formosa (4.2 broods per year) indicates that realized annual fecundity varies among *H. guttulatus* populations. Spawning frequencies are positively correlated with temperature in many marine fishes (Bone et al., 1995), including darters (Gale and Deutsch, 1985). Variation in spawning frequency among northern anchovy populations was also linked to energetic constraints (4–20 spawnings per year, Hunter and Leong, 1981). With an estimated mean spawning frequency of once per year and a shorter reproductive season, *H. guttulatus* in the Arcachon Basin may be more vulnerable to environmental stochasticity than the Ria Formosa population. Spawning that occurs multiple times within a reproductive season confers fitness benefits, particularly for small-bodied fishes inhabiting variable environments. By spawning fewer times in variable environments, the risk of catastrophic losses of eggs and larvae due to temporarily poor environmental conditions are increased (Nikolsky, 1963; McEvoy and McEvoy, 1992).

**Figure 4**

Within-year variation in the reproductive activity of *Hippocampus guttulatus* (long-snouted seahorse) in the Arcachon Basin from June to September 1952. The equation for the solid line fitted to the data from Boisseau (1967) is given. The dashed line is fitted to catch data from the Ria Formosa lagoon (see Fig. 3A).

#### Trends in reproductive activity

Peaks in the reproductive activity of male and female *H. guttulatus* in the Ria Formosa lagoon

corresponded to months with warmer water temperatures and higher primary and secondary production (Sprung, 1994a, 1994b), as observed in other fishes (Bye, 1984; Milton and Blaber, 1990). The duration of the reproductive season in the Ria Formosa lagoon (March–November) was similar to that reported for populations in southern Spain at a similar latitude (March–October, 36.7°N; Reina-Hervás, 1989) but was almost twice as long as in the higher latitude Arcachon Basin population (May–September, 44.7°N; Boisseau, 1967). Among-population differences in the duration of the reproductive season may be attributable to differences in temperature regimes (Robert et al., 1993) and photoperiod. Although Boisseau (1967) suggested that peaks in *H. guttulatus* reproductive activity occurred during full moons in the Arcachon Basin, there was no evidence of a correlation between lunar phase and reproduction in the Ria Formosa.

### Models for estimating realized annual fecundity

Neither assumption of the CR model (rapid remating and continuous reproduction) was met empirically for *H. guttulatus* (Table 1, Fig. 3). This means that accurate estimates of realized annual fecundity in other seahorse species of conservation concern (Foster and Vincent 2004; IUCN<sup>1</sup>) may require further biological research. Annual spawning frequency and realized annual fecundity may be overestimated by as much as 270% when the assumptions of rapid remating or continuous reproduction have not been met (Table 1). Assumptions inherent in the CR model may be more likely to hold for monogamous species with short interbrood intervals (e.g., *H. whitei*; Vincent and Sadler, 1995) that breed year round in tropical environments (e.g., *H. comes*; Perante et al., 2002). Incorporating an estimate of interbrood interval into the IR model produced intermediate estimates of annual spawning frequency. However, estimates of interbrood intervals required considerable effort because of the need to monitor changes in the reproductive activity of individually tagged seahorses. Use of the ISR model circumvented the need to directly estimate interbrood intervals (see “Material and methods” section) and led to unbiased estimates of spawning frequency for both males and females.

The success of the ISR model in predicting observed estimates of male spawning frequency and the number of days females spent preparing eggs indicates that this model, based on the spawning fraction method developed by Hunter and Leong (1981), may be broadly applicable to all organisms for which batch fecundity and the time to prepare or brood a clutch of eggs are known. This model is suitable for species with broods that can be readily and periodically surveyed by using either fisheries-independent catches or underwater visual census techniques. Although fisheries-independent collections were opportunistically used in this study to estimate brood sizes and the fraction of spawning males and females, reliable estimates of these values were also obtained nondestructively by caging brood-

ing males to count batch fecundity directly, and by monitoring changes in the fraction of spawning males and females over time with underwater visual censuses (Fig. 3). For species at risk that can be surveyed periodically during the reproductive season with non-destructive fisheries-independent sampling (e.g., underwater visual census), the generalized spawning fraction method becomes a potentially effective and appropriate means of estimating spawning frequency and realized annual fecundity.

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