Functional Ecology

British Ecological Society

Functional Ecology 2015, **29**, 1533–1542 doi: 10.1111/1365-2435.12465

Birth date predicts alternative life-history pathways in a fish with sequential reproductive tactics

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Summary

1. In species with plastic expression of alternative reproductive tactics (ARTs), individuals of the same sex, usually males, can adopt different reproductive tactics depending on factors such as body size.

2. The 'birth date hypothesis' proposes that condition-dependent expression of ARTs may ultimately depend on birth date, because individuals born at different times of the year may achieve different sizes and express different reproductive tactics accordingly. However, this has rarely been tested.

3. Here, we tested this hypothesis in a fish with ARTs, the peacock blenny (*Salaria pavo*). A long-term (6 years) mark–recapture study demonstrated that ARTs in the peacock blenny were sequential and that males may follow at least two alternative life-history pathways: a nest-holder pathway, in which males express the nest-holder tactic from their first breeding season onwards, and a parasitic pathway, where males reproduce on their first breeding season as sneaker males and subsequently as nest-holders.

4. We have found evidence of a birth date effect on the expression of ARTs in the peacock blenny. Males following the nest-holder pathway are born earlier and are larger at the first breeding season than males following the parasitic pathway, but they have similar growth curves.

5. The mechanisms underlying a birth date effect are far from clear and might be diverse. We have not found support for a mechanism of body size threshold triggering sexual maturation and subsequent ARTs. A mechanism of tactic determination that is strictly based on timing of first maturation is also unlikely.

6. A proxy of lifetime reproductive success shows crossing (body size associated) fitness curves for the two main life-history pathways.

Key-words: alternative life histories, alternative reproductive tactics, birth date effect, blenny, mark–recapture study, nest-holder, sneaker

Introduction

How evolution selects genotypes that are able to modulate phenotype expression according to environmental conditions, and in particular to social conditions, is one of the most exciting questions in biology today. Alternative reproductive tactics (ARTs) are excellent models to study condition-dependent phenotype expression, although other forms of determination of ARTs exist. ARTs allow different individuals within the same population to maximize their reproductive success through the expression of different behaviours coordinated with other traits, often morphological traits (Brockmann 2001; Taborsky, Oliveira & Brockmann 2008). In condition-dependent ARTs, different

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individuals (or the same individual at different moments) express the tactic that best suits its own or surrounding conditions. Therefore, for each of the tactics, the reproductive success is expected to be maximum under the conditions in which the tactic is usually expressed. Each of the tactics presents therefore a distinct fitness curve, that is, the relationship between environmental (or individual) conditions, and the reproductive success has a different form for each of the different tactics. In such a way that, for one tactic, the maximum reproductive success is achieved under certain conditions, but for the alternative tactic, the maximum reproductive success is achieved under different conditions. Therefore, we expect the fitness curves of the different tactics to cross (Brockmann & Taborsky 2008).

If conditions triggering tactic expression have been frequently investigated, the same cannot be said for its phenology, despite obvious examples of the influence of season in phenotype expression (e.g. in insect camouflage). Here, we specifically test the birth date hypothesis (Taborsky 1998), which proposes a seasonal effect on the expression of ARTs.

In resource-based mating systems, body size is vital for the successful defence of reproductive resources. In such systems, ARTs may be associated with body size, with large males reproducing by gathering and defending resources, and smaller males, unsuccessful at resource defence, parasitizing the investment of the large males and reproducing by cuckoldry. Associations between ARTs and body size are therefore common, and body size is frequently a trigger for tactic expression (Emlen 1997; Tomkins 1999; Moczek & Nijhout 2002). Density and the relative frequency of each alternative phenotype are also common cues for conditional tactic expression (Radwan 2001; Leary, Garcia & Knapp 2008). The birth date effect has been proposed as a mechanism for tactic determination in species where body size is relevant for reproductive success and where ARTs are condition dependent (Taborsky 1998). In species that have long breeding seasons and inhabit seasonal environments, individuals born earlier in the season should not only enjoy a longer growing period until the next breeding season, but should also meet different environmental conditions. Consequently, due to different growth periods and due to different growth rates, individuals born at different periods of the same year are expected to have different body sizes at the start of the breeding season. According to this hypothesis, surpassing a minimum threshold of body size or, alternatively, a threshold of growth rate will trigger sexual maturation and an associated ontogenetic switch that will determine the development of individuals maturing at different times into different reproductive phenotypes, different ARTs. Bass (1996) has proposed an alternative mechanism of ARTs expression, associated with age at sexual maturation. Males may mature gonads and other morphological structures and physiological pathways simultaneously, but when sexual maturation is precocial, it may precede maturation of remaining structures and avoid for instance the expression of secondary sexual characters. This developmental asynchrony has been called heterochrony.

The influence of the timing of birth on life-history traits from survival and growth and on timing of maturation and reproductive success has been widely demonstrated in several taxa (Price, Kirkpatrick & Arnold 1988; Schultz 1993; Cargnelli & Gross 1996; Dawson & Clark 2000; Descamps *et al.* 2006; Divino & Tonn 2007). However, not much is known about its effects on the incidence of different reproductive phenotypes, and most studies are restricted to sex ratio adjustments that include diverse mechanisms of phenotype determination (Dijkstra, Daan & Buker 1990; Schultz 2008). The 'birth date hypothesis' in the context of ARTs has only rarely been tested. Pastres *et al.* (2002) modelled the growth of different life-history pathways of the black goby, *Zosterisessor ophiocephalus*, based on otolith analysis and found support for the 'birth date hypothesis'.

Within condition-dependent ARTs, species presenting sequential patterns of tactic expression are ideal models to study phenotype expression both from a functional and from a mechanistic point of view. Sequential ARTs represent complex and well-coordinated mechanisms of trait expression that can be analysed within the same individual, therefore eliminating noise from different genetic backgrounds. One such species is the blenny *Salaria pavo* (Risso, 1810). This fish has been thoroughly used as model for ARTs, and yet precise knowledge regarding some fundamental features of its ARTs pattern of expression is still missing.

The Portuguese population of *S. pavo* inhabiting the coastal lagoons of Algarve presents ARTs that are characterized by large nest-holder males and small sneaker males that parasitize the reproductive investment of nest-holders by surreptitiously fertilizing eggs inside their nest. The fact that there is almost no overlap in body size class between sneakers and nest-holders suggests sequential ARTs (Gonçalves *et al.* 1996). Additionally, immature males of the same age class as sneakers have also been described (Gonçalves *et al.* 1996), suggesting that males may follow one of three different life-history pathways: the nest-holder pathway – males reproduce directly as nest-holders in their first breeding season and maintain that phenotype throughout their lives; the parasitic pathway – males reproduce as sneakers in their first breeding season and as nest-holders in the following seasons; and the immature pathway – males postpone reproduction in the first breeding season and adopt the nest-holder tactic either earlier or at a larger body size in the following breeding season, therefore increasing their chances of successfully reproducing as nest-holders.

In this paper, we present a long-term mark–recapture study that tests the birth date effect and its influence on the determination of the sequential pattern of ARTs expression of the peacock blenny. This study also explores

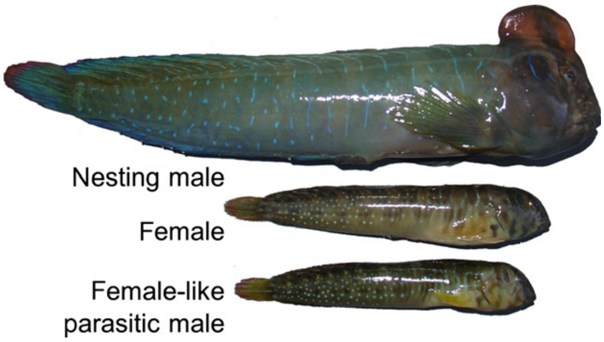

Fig. 1. A nesting male, a female and a female-like sneaker male of peacock blenny, *Salaria pavo*.

the conditions under which the different pathways are expressed and its functional value.

Materials and methods

STUDY SPECIES

The *S. pavo* population studied inhabits the intertidal zone of a coastal lagoon in the south of Portugal. In this population, two reproductive phenotypes are present: nest-holders and sneakers (Gonçalves *et al.* 1996; Saraiva *et al.* 2012). Nest-holder males are larger and older, and express a suite of secondary sex characters, namely a head crest and an anal gland that produces a sex pheromone (Gonçalves *et al.* 1996; Serrano *et al.* 2008). Nest-holders defend a nest to which females are attracted, and they provide parental care to eggs that several females lay in their nests. Sneaker males are smaller and younger than nest-holders and lack secondary sex characters (Fig. 1). Sneakers are sexually mature and mimic the courtship behaviour of the females in order to approach nest-holder males and fertilize part of the eggs that the nest-holders will attend. Additionally, immature males of the same age class as sneakers have also been described (Gonçalves *et al.* 1996), suggesting that males may follow one of the three different life-history pathways described above (see Introduction): a parasitic pathway, a nest-holder pathway or an immature pathway.

FIELD SITE AND SAMPLING METHODS

The population studied inhabits the Culatra island (36°59′N, 7°51′W), Parque Natural da Ria Formosa (Ramsar RS212), Algarve, Portugal (for a detailed description of the habitat, see Almada *et al.* 1994). In this region, clam culturists use bricks and other debris to delimit their fields, and *S. pavo* males use these structures as shelters and nest sites. A 55-m transect composed of 80 brick structures was placed in the intertidal area. This transect was monitored from May 2000 to August 2006. A total of 90 censuses were conducted in this transect in regular intervals of time: once every 2 weeks during the breeding season (from the beginning of May to middle September); and once a month, outside of the breeding season. The census was conducted during low tide, when the area is emerged and fish can be easily collected. Once captured, individuals were anaesthetized with MS-222 (tricaine methanesulphonate; Pharmaq Ltd, Oslo, Norway, dilution 1 : 10 000 sea water) and marked with a passive transponder (passive integrated transponder tag: ID100A, Trovan, Aalten, the Netherlands; 11 × 2·1 mm, maximum reading distance 0·5 m with portable reader LID-500). The device was inserted in the abdominal cavity of the fish. The minimum body size that can accommodate the tagging device is 5·2 cm of total length (TL). The sex and morphotype of each individual, its location and morphological characteristics were registered in each census. After recovery from anaesthesia in an aerated container, marked fish were returned to their original crevices.

During the monitored period, 1182 different individuals were marked, and 836 fish were sighted but not marked. Of the marked fish, 660 individuals were males, and for males, the recapture rates were high, with 54·5% of the males being recaptured at least once (2–5 observations: 248 males; 6–10 observations: 55 males; 11–20 observations: 39 males; 20–41 observations: 19 males). The male with the highest recovery rate was captured 41 times, from July 2000 to June 2003 (estimated longevity: 4 years). Due to their small body size (TL < 5·2 cm, *n* = 533), or because they were sighted but could not be captured (*n* = 190), or for other reasons (e.g. signs of sickness or extreme pregnancy, *n* = 113), 836 individuals could not be marked.

The morphological measures taken included TL, body height (measured at the level of the insertion of the pelvic fins) and maximum head height, all measured to the nearest millimetre. The weight was measured with a spring balance (Pesola, Switzerland) to the nearest gram. Individuals were sexed according to the genital papilla: females present an atrium that precedes the opening of the papilla, whereas the males have a tubular and protuberant papilla. Male morphotypes were further classified based on the development of secondary sexual characters and the presence or absence of milt during the breeding season. The relative development of male secondary sexual characters, head crest and anal gland, was classified using an ordinal scale from 0 (absent) to 9 (maximum development). We validated this scale by correlating, in a subset of fish, values obtained on this ordinal scale with continuous values measured with a calliper. The ordinal classification of the relative development of the head crest was highly correlated with the maximum head height/body height ratio (Spearman's correlation, $r_s = 0{\cdot}927$, $n = 363$, $P < 0{\cdot}001$). Likewise, the ordinal classification of the relative development of the anal gland was highly correlated with the area of the anal gland/TL ratio ($r_s = 0{\cdot}873$, $n = 68$, $P < 0{\cdot}001$). The area of the anal gland, which has an elliptical shape, was calculated as follows: π × Length/2 × width/2. Values below 3 correspond to absent or vestigial sexual characters, typical of females and sneakers. Therefore, males were classified as nest-holder if the relative sizes of the head crest and anal gland were ≥3 on our ordinal scale. Sneaker males were classified as such if the relative development of the head crest and/or of the anal gland was smaller than 3. Nonreproducing or immature males are morphologically similar to sneakers but do not have mature gonads. Only sneakers release milt when their abdomen is pressed (Oliveira *et al.* 2001), this criterion was applied to discriminate sneakers from immature males. This criterion was validated by the inspection of testes development after testing for milt release in a subset of sneaker and immature males (*n* = 12 per group). Nest-holder males also did not release visible amounts of milt.

The number of eggs inside each nest was monitored and estimated based on Gonçalves *et al.* (2002), who describes eggs as being attached to the surface of the nest in a single layer with an average density of 80 eggs per cm^2.

ANALYTICAL PROCEDURE

Only males with TL ≥5·2 cm could be marked; therefore, the size class 5·2–7·2 cm was considered the recruiting class. The onset of recruitment of the smallest observable class (3·2–5·2 cm) and of the recruiting class was identified as the largest percentage increase in frequency of those classes. As expected, the onset of recruitment of the recruiting class followed closely the onset of recruitment of the smallest observable class, which is also the one presenting minimum age overlap (Fig. S1, Supporting information). Each cohort

Table 1. Growth parameters and estimated birth date of the males of the peacock blenny following different life-history pathways. The cohorts 2000 through 2004 were used for the analysis

Life-history pathway	$TL_\infty \pm SE$	$K \pm SE$	φ'	Birth date ± SE	r^2	N
Nest-holder	13·80 ± 0·42 cm	1·03 ± 0·12	2·293	15 May ± 14·6 days	0·86	87
Parasitic	13·42 ± 0·48 cm	0·92 ± 0·11	2·220	28 Aug ± 28·3 days	0·90	84
Transient	12·89 ± 1·23 cm	1·21 ± 0·38	2·303	15 Jul ± 12·8 days	0·88	13

L_∞, asymptotic length; K, growth constant, the rate at which TL approaches L_∞; t_0, age a fish would have had at TL = 0; φ', growth performance index; r^2, growth curve fit; TL, total length.

was then defined as the group of all the males that reached the recruitment size between the beginning of the recruitment to the sample and the beginning of the recruitment of the following year. Males of the same age could be identified in this way and assigned to the same cohort but, although the growth trajectories could be observed from the moment the males were marked (Fig. S2, Supporting information), their age would have to be confirmed by estimating a general growth curve of all males and estimating age at minimum recruitment size (TL = 5·2 cm).

Growth curves were estimated on the software FISAT II (FAO/ICLARM Stock Assessment Tools 2005; Gayanilo, Sparre & Pauly 2005) using growth increment data composed of the first and the last TL measurements of each male. Growth curves followed the Von Bertalanffy model and were fitted using the Appeldoorn's method. The Von Bertalanffy model is $TL = L_\infty(1 - e(-K(t - t_0)))$, where L_∞ is the asymptotic length, that is the maximum length the fish would reach if living indefinitely; K is the growth constant, the rate at which TL approaches L_∞; and t_0 is the age a fish would have had at length zero (TL = 0). Because most individuals were indeed captured and marked before their first breeding season, the subsequent observed morphological development allowed the clear identification of the complete life-history pathway of most males (i.e. the complete chronological sequence of reproductive tactics expressed by each male, Table 1). Specific growth curves were then estimated for each of the identified life-history pathways. Observed growth trajectories are truncated at the first and last year of sampling. To minimize the impact of truncated data, only males of the cohorts 2000–2004 were considered for the analysis of the life-history pathways. Still, within these limits, 16 of the 660 tagged males could not be attributed to any particular cohort due to overlap of growth trajectories (empty circles in Fig. S2, Supporting information) and these were also excluded from the analysis. Differences between growth curves of different life-history pathways were assessed by comparison with the confidence intervals of estimated parameters of the Von Bertalanffy curve and through growth performance indices, $\varphi' = \log K + 2\log L_\infty$ (Pauly & Munro 1984). To determine the average birth date of each life-history pathway, their estimated growth curves were fitted to the time of year by minimizing the sum of squared residuals. All cohorts were pooled as if fish had all been born in the same year. Confidence intervals of 95% [average TL ± 1·96 standard error (SE) of residuals] were computed for growth curves and consequently for birth dates, assuming a normal distribution of residuals. To ensure independence, only one randomly chosen sampling point per male was used in this analysis. This provided a conservative estimate of confidence intervals for average birth date. The same data were used to compare the position of the different growth curves. An ANOVA was conducted to test differences between the residuals of male body size (TL) of each pathway to the same reference growth curve that of the males following the nest-holder pathway. This allowed for the detection of significant differences in the position of the growth trajectories and, therefore, between the birth dates of the male pathways.

Differences between the timing of the onset of the nest-holder tactic (onset of the expression of the secondary sexual characters) in the different life-history pathways were analysed through a chi-square test. Analysis of variance was also used to test differences in the observed growth rates of first-year males following different life-history pathways. In this case, pathway and season were tested as fixed effects and controlled for body size (TL) as random factor.

Survival rates from the first to the second breeding season of males following the main life-history pathways were determined with the program 'Mark-version 7.1-Mark and recapture parameter estimation' (Gary White, Department of Fish, Wildlife and Conservation Biology, Colorado State University, USA) using the Cormack–Jolly–Seber method on live recapture data (Cooch & White 2010). The sampling points within each of the different breeding seasons were pooled and treated as a single sampling point per year, originating a model with six sampling occasions, that is six breeding seasons. The general model, an age model, with life-history pathway and body size (small: TL < 7·9 cm vs. large; TL ≥7·9 cm) as factors and all two-way interactions, was sequentially reduced and compared to the best fitting (lower Akaike's information criterion (AIC) after correction for c-hat) reduced model through a LR test. Median c-hat, χ^2/d.f., a parameter that estimates overdispersion, was determined for the general model to verify general fit (Cooch & White 2010).

A hierarchical linear mixed model tested differences in relative body size of the life-history pathways prior to start of the breeding season. Our GLMM had pathway as a fixed, level 1 effect and year as level 2 subject, therefore removing the effect of yearly differences in body size distribution, which allows the analyses of relative body size across years. This model was compared by a LR test with a simpler model that did not include the intercept of year. The potential effect of density, tactic frequency and number of older males on the frequency of tactics within each new cohort was tested by regression analyses.

All linear models and chi-square tests were conducted on IBM SPSS Statistics for Windows, version 20.0. IBM Corp. Released 2011 (IBM Corp., Armonk, NY, USA). All tests were two-tailed and significance level was set to 0·05.

Finally, a proxy for the reproductive success of males whilst nest-holders were calculated as the total number of eggs present in nests of nest-holders throughout their lives, assuming equal first-year survival and ignoring sneaker paternity, was known to be low (on average 4·66% ± SD = 6·53% of the eggs inside the nest are not sired by the nest owner, S. Cardoso, D. Gonçalves, R.F. Oliveira, unpublished data). In order to calculate differences in the reproductive success of the different pathways, we have used a model of zero-inflated negative binomial since the majority of males never reproduced as nest-holders. We have tested both the effect of the pathway and of its interaction with body size using R 2.15.2. (packages: 'Rcmdr version 1.9-2', 'pscl', 'MASS').

Results

LIFE-HISTORY PATHWAYS

Recruitment to the sample varied with year and started between October and January (Fig. S2). General growth was well described ($r^2 = 0{\cdot}87$) by a Von Bertalanffy curve, $\mathrm{TL} = \mathrm{TL}_\infty\ (1 - e(-K(t - t_0)))$, with parameters $\mathrm{TL}_\infty \pm \mathrm{SE} = 14{\cdot}20 \pm 0{\cdot}23$ and $K \pm \mathrm{SE} = 0{\cdot}80 \pm 0{\cdot}04$, $n = 362$. Average birth date (15 June) fell within the breeding period of the year of recruitment, hence confirming that recruiting individuals are the young of the year (0 age class). Within each cohort, three life-history pathways were observed (Table 1): (i) the nest-holder pathway, which consisted of the larger individuals that expressed the nest-holder tactic (i.e. males with secondary sexual characters) from their first year of age onwards; (ii) the parasitic pathway, which was composed of smaller males lacking secondary sexual characters that reproduced as sneakers in their first year and switched to the nest-holder morphotype after their first breeding season; and (iii) the transient pathway, an uncommon pathway, composed of males that express the sneaker tactic only for a short period of time and that switched to the nest-holder tactic still within their first breeding season. Very few males were not mature during the breeding season and these had very low recapture rates. Hence, no substantial information could be gathered to confirm the existence of a fourth immature pathway, in which males would postpone reproduction during their first year of life.

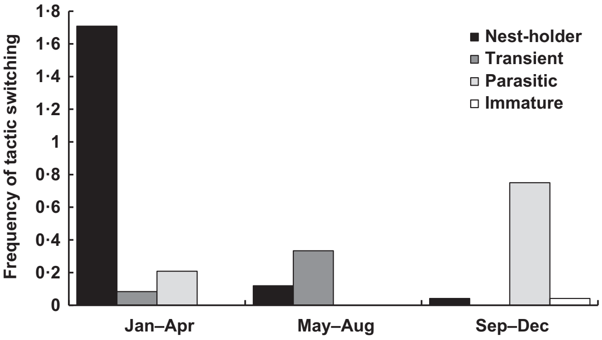

Fig. 2. Switch to nest-holder morph by males following different life-history pathways, across the year. The switch to the nest-holder morph corresponds to the onset of the expression of the secondary sexual characters. The breeding season corresponds roughly to the months of May to August. The data refer to the frequency of observed switches per sampling point and to the cohorts 2000 through 2004.

The distribution of the onset of the expression of secondary sexual characters (i.e. the onset of the expression of the nest-holder morph) was bimodal and associated with the life-history pathway [$\chi^2 = 120{\cdot}988$, degrees of freedom (d.f.) = 8, $P < 0{\cdot}001$]. Males following the Nest-holder Pathway expressed the nest-holder morph before the breeding season, whereas those males following the parasitic pathway switched to the nest-holder morph after their first breeding season (Fig. 2). Thirteen males following the transient pathway initiated the expression of the nest-holder morph within the breeding season, mostly around June. Only one male classified as immature was observed to initiate the expression of the nest-holder morph, and similarly to males following the parasitic pathway, this occurred immediately after the breeding season (Fig. 2).

GROWTH AND BIRTH DATE

Males of different pathways presented similar growth curves. The growth curves estimated for each pathway fitted the data well (Table 1), except for the immature pathway, for which no fit was determined (only five paired data points were available). The confidence interval of the estimated parameters overlapped and the growth performance indices, φ′, were similar in all groups. Therefore, the form of the growth curves was similar between pathways (Table 1). However, the position of the growth curve of the parasitic pathway differed from that of the transient and the nest-holder pathways (ANOVA: $F_{2,263} = 92{\cdot}664$, $P < 0{\cdot}001$; LSD pairwise comparisons Nest-holder vs. Transient $P = 0{\cdot}128$, Nest-holder vs. parasitic $P < 0{\cdot}001$, Parasitic vs. Transient $P < 0{\cdot}001$, test applied to the residuals of each group to the reference growth curve, i.e. the nest-holder pathway). Consequently, the estimated average birth dates also differed between these male classes, with males following the Nest-holder and the transient pathway hatching earlier than males of the parasitic pathways (Fig. 3, Table 1). The observed absolute growth during the

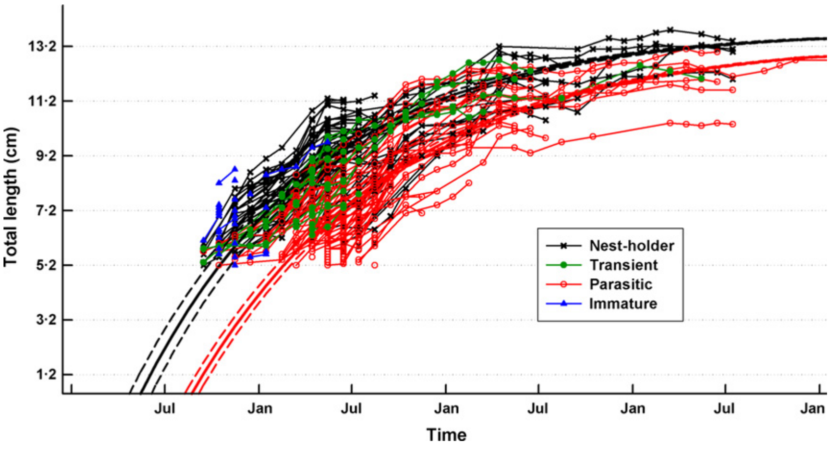

Fig. 3. Observed and estimated growth of males following different life-history pathways. Thin lines represent the observed growth and thicker lines represent the estimated growth curves. Estimated birth date is represented by the interception of the estimated growth curves with the x-axis [total length $(\mathrm{TL})_{\mathrm{birth}} = 0{\cdot}5$ cm] and the dotted lines represent the standard error. Pooled data from the cohorts 2000 to 2004 are presented.

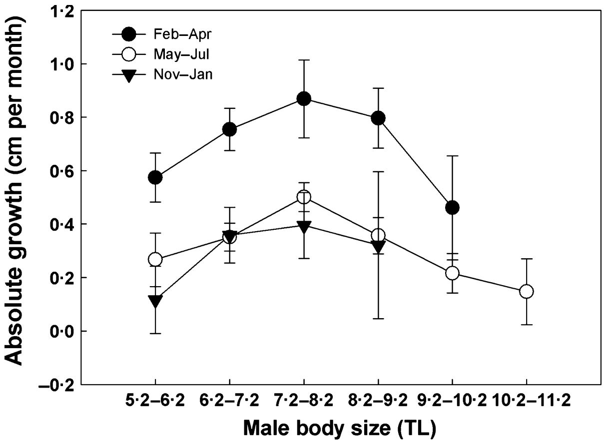

Fig. 4. Absolute monthly growth of males during their first year, as function of body size and season. Growth curves did not differ between life-history pathways and thus are not depicted in the figure.

1st year of life did not differ between the nest-holder and the parasitic pathways when controlled for size, as random factor, and season, as fixed factor (Fig. 4, ANOVA Pathway $F_{1,186} = 1{\cdot}448$, $P = 0{\cdot}230$; Season $F_{2,186} = 19{\cdot}276$, $P < 0{\cdot}001$; Pathway × Season $F_{2,186} = 0{\cdot}378$, $P = 0{\cdot}686$; TL as random factor $F_{5,186} = 16{\cdot}285$, $P = 0{\cdot}008$). In fact, only season and TL predicted growth, and the reduced model with only these two factors presented the best fit (AIC = 69·936, BIC = 76·472 against AIC = 84·694, BIC = 91·320 for the initial model). Two points of slower growth were observed in winter, from November to January, and during the summer period, from May to July, which corresponds approximately to the breeding season (Fig. 4).

Prior to the first breeding season, males following the nest-holder pathway were larger than those following the parasitic pathway (*t*-test: $t = 8{\cdot}95$, d.f. = 90, $P = 0{\cdot}000$, Table 2), but the data fitted better to a hierarchical model controlling for year intercept (initial reduced model BIC: 243·452 vs. year intercept model: BIC: 234·723, LR test = 13. 229, d.f. = 1, $P < 0{\cdot}005$). Therefore, differences in relative body size are better predictors of life-history pathways than differences in absolute body size. Moreover, the incidence of the nest-holder tactic in the new cohorts was negatively correlated with the number of males of older cohorts present in the population ($r_s = -0{\cdot}975$, $n = 5$, $P = 0{\cdot}005$), but not to density ($r_s = 0{\cdot}700$, $n = 5$, $P = 0{\cdot}233$) or to tactic frequency ($r_s = -0{\cdot}9$, $n = 5$, $P = 0{\cdot}083$), although the latter was only marginally nonsignificant.

SURVIVAL AND REPRODUCTIVE SUCCESS

Both life-history pathway and body size at first breeding season and their two-way interactions with age influenced survival rate from the first to the second breeding season (Fig. 5). Our general model fitted the data well, with median c-hat ± SE = 1·135 ± 0·085, but excluding the

Table 2. Life-history pathways followed by males of the peacock blenny and corresponding total length (TL) (mean ± SE) observed at different ages

Life-history pathway	Before 1st breeding season (January–April)	1st breeding season (age 0+/1)	2nd breeding season (age 2)	3rd breeding season (age 3)	4th breeding season (age 4)	TL at onset of Nest-holder tactic
Nest-holder	TL = 8·1 ± 0·12 cm *N* = 58	TL = 8·8 ± 0·12 cm Nest-holder *N* = 102	TL = 11·8 ± 0·27 cm Nest-holder *N* = 11	TL = 13·0 ± 0·22 cm Nest-holder *N* = 5		8·4 ± 0·13 cm *N* = 41 Before first breeding season
Parasitic	TL = 6·4 ± 0·13 cm *N* = 34	TL = 6·8 ± 0·08 cm Sneaker *N* = 139	TL = 11·1 ± 0·2 cm Nest-holder *N* = 19	TL = 12·3 ± 0·23 cm Nest-holder *N* = 6	TL = 12·9 cm Nest-holder *N* = 1	9·3 ± 0·23 cm *N* = 20 After first breeding season
Transient	TL = 7·0 ± 0·22 cm *N* = 8	TL = 8·4 ± 0·25 cm Sneaker → → Nest-holder *N* = 13	TL = 11·9 ± 0·33 cm Nest-holder *N* = 3	TL = 12·0 cm Nest-holder *N* = 1		8·5 ± 0·22 cm *N* = 13 During first breeding season
Immature	TL = 6·1 cm *N* = 1	TL = 6·8 ± 0·19 cm Immature *N* = 26				9·2 cm *N* = 1 After first breeding season

two-way interaction between life-history pathway and body size did not significantly improve the fit [LR test (general model vs. reduced model) = 0·000, d.f. = 1, $P > 0{\cdot}1$.]; hence, the main effects model was the most parsimonious.

A zero-inflated binomial model revealed a significant effect of life-history pathway on reproductive success. Only the zero inflated part of the model was significant, therefore suggesting that males following the nest-holder pathway had a higher probability of successfully reproducing as nest-holders than males following the parasitic pathway (zero-inflated model binomial with logit link Z value = 2·846, P = 0·004, estimated effect = 0·386; count model neg bin with log link Z value = 1·003, P = 0·316 estimated effect = 0·386; log likelihood = −533·7, d.f. = 5). Within the nest-holder pathway, larger males were more likely to reproduce (zero-inflated model binomial with logit link Z value = −2·065, P = 0·039, estimated effect = −0·577; count model neg bin with log link Z value = 0·268, P = 0·789, estimated effect = 0·073; log likelihood = −300·3, d.f. = 5). Within the parasitic pathway, size did not significantly affect the number of eggs sired by the males, but a marginally nonsignificant effect of size was detected on the probability of reproducing (zero-inflated model binomial with logit link Z value = −0·4942, P = 0·06941, estimated effect = −1·816; count model neg bin with log link Z value = −0·2778, P = 0·782, estimated effect = −0·08232; log likelihood = −228·9, d.f. = 5; Fig. 6).

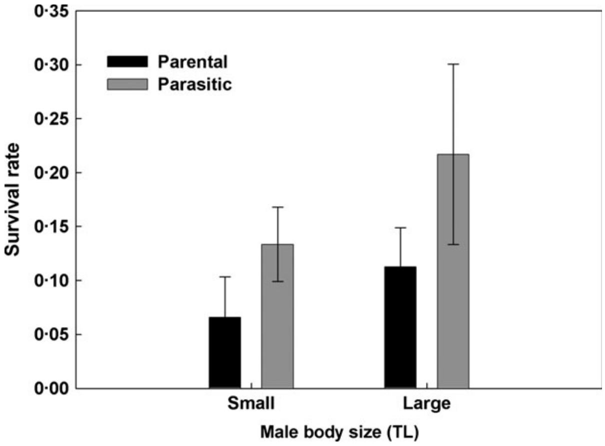

Fig. 5. Mean survival rate from the first to the second breeding season of small and large males of the peacock blenny following different life-history pathways. Errors bars represent 95% confidence interval.

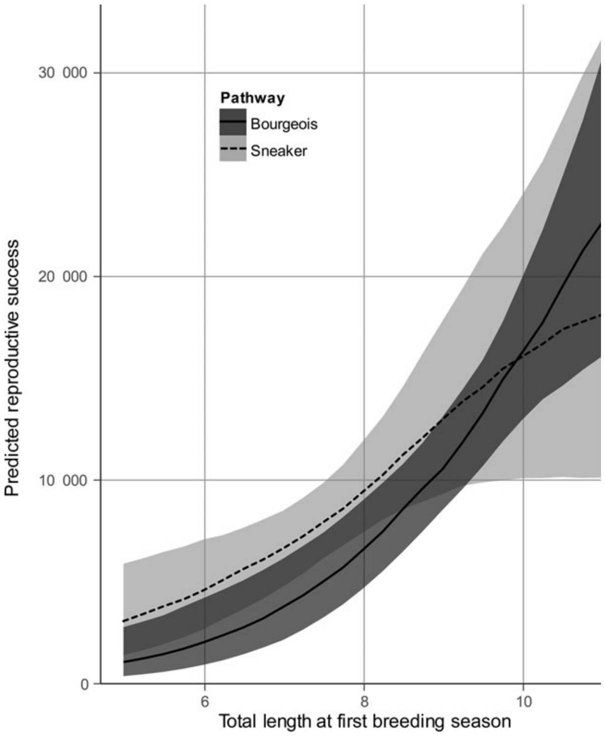

Fig. 6. Expected lifetime reproductive success of males of the peacock blenny according to body size. Grey areas represent quartiles of bootstrapped predicted values under zero-inflated negative binomial model.

Discussion

The present study supports a birth date effect (Taborsky 1998) in the expression of ARTs in the peacock blenny. The study confirms that the ARTs are sequential in the studied species and that males follow different life-history pathways associated with the timing of birth. Three alternative life-history pathways were identified. In the Nest-holder Pathway, males are born at the start of the breeding season and express the nest-holder tactic at 1 year of age and throughout their lives. The parasitic pathway is formed by males that are born later in the same season. Males following this parasitic pathway reproduce as sneaker in their first year of life, postpone the onset of the expression of sexual characters until after the breeding season and reproduce as nest-holders in subsequent years. A third, less represented pathway was also identified in the present study, the transient pathway, which is composed of males born at mid-breeding season, and that reproduces shortly as sneakers and switch to nest-holders at 1 year of age, still within their first breeding season.

At 1 year of age, males following the parasitic pathway are smaller than the males of the same cohort that reproduce as nest-holders and that form the nest-holder pathway. This difference in body size is, however, not the result of different growth rates, as proposed by the birth date hypothesis. Contrary to the results of Pastres *et al.* (2002), which also describe a birth date effect in the determination of ARTs in the grass goby (*Zosterisessor ophiocephalus*), we have found no support for different growth rates between the two life-history pathways. Because the breeding season extends for approximately 4 months, early-born males have significantly more time to grow than later born males until they reach the first breeding season (age 0+/1). This seems to be the main reason for the body size differences between males of the parasitic and nest-holder pathways in their first year of life. Whilst 1-year-old males differ in their reproductive tactic, 2-year-old and older males all adopt the nest-holder tactic. ARTs in *S. pavo* are therefore plastic and alternative life-history pathways also occur.

The 'birth date hypothesis' also suggests that either body size or growth rate may determine the timing of sexual maturation and associated tactic expression. In the pathways identified in our study, all males mature simultaneously, in the first breeding season, but at different body sizes. Therefore, we have found no support for the existence of a body size threshold to trigger sexual maturation and subsequent tactic development. However, because the two pathways mature simultaneously and therefore at different ages (a few months of difference), we may consider that tactic expression is associated with age at first maturation. In the plain midshipman, it has been proposed that different ages of sexual maturation could lead to heterochrony, in which conventional males are mature and express typical male traits but sneakers, maturing at an earlier age, have not yet developed the necessary physiological pathways to express typical male structures (Bass 1996). A similar hypothesis could be proposed for the peacock blenny. However, Fagundes *et al.* (2012) demonstrated that the differentiation of the secondary characters typical of nest-holder males is dependent on the dominance status of the juveniles and their relative body size within a social group, rather than on the absolute body size of males. Accordingly, in the present study, body size is shown to differ between males following the different pathways, but this difference is better explained when considering year variation, thus suggesting that pathways are best described by their relative differences in body size rather than by fixed absolute body size values. Further support for the role of relative body size on tactic expression comes from the negative association found between the number of older, thus larger, males in the population and the incidence of the nest-holder pathway in the new cohorts. This is not compatible with a mechanism of tactic determination strictly based on age at first maturation. Instead, it is more likely that birth date creates a temporal pattern that predictably influences the social context that males meet; it determines male's relative body size and associated dominance status, which in turn influences tactic determination.

Great asymmetry in male reproductive success and crossing fitness curves, in which the maximum reproductive success for different conditions is achieved by different reproductive behaviours, have been proposed as a drive for ART's appearance and maintenance (Brockmann & Taborsky 2008). Moreover, in coarse-grained environments, where cues for reproductive success are available, condition-dependent developmental tactics are expected to arise (Shuster & Wade 2003). In the peacock blenny, dominance status and access to reproductive resources, specifically nests, are expected to change predictably with body size. Our data show that lifetime reproductive success as nest-holder is positively associated with body size at first breeding season and that this relationship is different for each of the pathways. The reproductive success of the Nest-holder Pathway shows a stronger positive association with body size at first reproduction than the parasitic pathway, for which, in fact, the association is marginally nonsignificant. Our measure of lifetime reproductive success is, however, only an approximate measure. First, we assume no differential survival until the first breeding season between the different pathways – a common but unverified assumption in many of these studies. Secondly, the reproductive success of the nest-holder pathway is overestimated in our study, for we ignore the eggs that are actually fertilized by sneakers, although present in the nests of nest-holders. We consider, nevertheless, that this measure is a good approximation of the total reproductive success of both pathways. Whereas for the Nest-holder phase the reproductive success is overestimated for both pathways, it is underestimated for the sneaker phase of the parasitic pathway. However, if size has any influence on the fitness of sneakers, we expect it to have a negative effect. Males mimic females partially through small body size, and larger sneaker males encounter more aggression from nest-holder males than do smaller ones (Gonçalves *et al.* 2005). As such, if we would consider the reproductive success of the sneaker phase, we would only reduce the slope of the fitness curve of the parasitic pathway, by increasing its reproductive success more for smaller than for larger body sizes. Hence, our test is a conservative one. Moreover, within nests, an average of 4·7% of the eggs are sired by sneakers, which can be considered a negligible contribution for the reproductive success of the nest-holder pathway and even more so for that of the parasitic pathway, since there are usually more sneakers than nest-holders on each breeding season.

Survival from the first to the second breeding season also increases with body size in both pathways. Nevertheless, the parasitic pathway has a higher survival rate than the nest-holder pathway. So, if a small body size significantly compromises both the survival and the reproductive success of the nest-holder pathway, reproducing as a sneaker (i.e. following the parasitic pathway) may be a more successful option for small males. Although the reproductive success of sneakers is low, their probability of reaching the second breeding season (and reproducing then as a larger Nest-holder) is higher than that of nest-holders.

The fact that males of the transient pathway, born at mid-breeding season, reproduce shortly as sneakers and switch to nest-holders still within their first breeding season, at 1 year of age, suggests that although the switch to the nest-holder morph is suppressed during the breeding season, a certain level of plasticity is still present at this point. In fact, in experiments conducted with this species in large outdoor pools with a surplus of nests, larger sneakers were observed to switch and successfully reproduce as nest-holders in <2 weeks and still within the period of the breeding season (R. Oliveira, E. Barata, A. Canário, R. Serrano, J. Saraiva, unpublished data). These observations also agree with a social effect on tactic determination, rather than a strict age effect on timing of sexual maturation and morphological development.

A hypothetical fourth pathway, the immature pathway, in which small males could postpone reproduction during the first breeding season and allocate resources into growth has not been confirmed. In previous studies, immature males have been observed during the breeding season (Gonçalves *et al.* 1996). In our study, however, they were uncommon during the peak of the reproductive season, when they can be distinguished from sneakers, and they were rarely ever recaptured. It is possible that such immature males avoid our sampling area, which is a breeding area, during the breeding season and find shelter elsewhere, far from competition for space and resources that may compromise their survival. This may explain the low frequency of immature males in our data set. Alternatively, males classified as immature males might be sneakers outside their reproductive period, either before maturing or after having reproduced. Immature males may also constitute a part of the unidentified males that recruit to our sample after the breeding season, therefore concealing their initial reproductive tactic. Hence, our data set does not allow us to definitively confirm or discard the existence of a fourth, immature pathway.

In conclusion, we found support for the 'birth date hypothesis' in the context of ARTs and confirmed that ARTs in the peacock blenny are sequential and irreversible, as suggested by previous studies It should also be noted that parental effects, such as choice (or constraints) of reproduction time, may play an important role on the birth date effect and should therefore be investigated in future studies.

Acknowledgements

The authors would like to thank the Ria Formosa Nature Park for providing logistic support and allowing the study to be conducted in the nature reserve area, and the inhabitants of the Ilha da Culatra for their continuous support and generous hospitality during the years of the study. We would also like to thank Ricardo Matos, Luís Carneiro, Magda Teles and João Alpedrinha for field assistance. This study was funded by sequential research grants of R.F.O., namely grants POCTI/BSE/38395/2001, PTDC/MAR/71351/2006, EXCL/BIA-ANM/0549/2012 and the R&D Units Pluriannual Program (R&D unit 331/2001) from the Portuguese Foundation for Science and Technology (FCT). During the course of this project, T. Fagundes was being supported by an FCT fellowship (SFRH/BD/6502/2001), J. Saraiva by a PhD fellowship (SFRH/BD/10764/2002) and A.F.H. Ros by a postdoctoral fellowship from FCT (SFRH/BPD/7143/2001). The procedures used in this study comply with the 'Principles of animal care', publication No. 86-23, revised 1985, of the National Institutes of Health, USA, and with the current laws of Portugal.

Data archiving

Data are deposited in the Dryad Digital Repository: doi:10.5061/dryad.6t9p3 (Fagundes *et al.* 2015).

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Received 5 July 2014; accepted 10 April 2015
Handling Editor: Jennifer Grindstaff

Supporting Information

Additional Supporting information may be found in the online version of this article:

Fig. S1. Monthly size distribution (TL) of male and juvenile peacock blenny, corrected for the number of samples conducted on each month.

Fig. S2. Observed growth trajectories of males attributed to different cohorts.