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Size-independent age effects on reproductive effort in a small, short-lived fish

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SUMMARY

1. Age-related changes in reproductive effort have been predicted by theoretical models and observed in a wide range of organisms. However, for indeterminate growers such as fish, an allometric relationship linking gonad weight to body size is commonly observed. There is often a positive linear relationship when these variables are log-transformed, which by implication reduces the influence of age on reproductive effort.
2. Contrasting with this usual pattern, we report a nonlinear relationship between gonad weight and fish size (after log-transformation) in mosquitofish (*Gambusia holbrooki*), clearly resulting from age changes. The declining rate of increase of gonad mass as a function of body size revealed a higher reproductive effort for younger individuals relative to size.
3. This size-independent age effect on reproductive effort was predicted based on previous studies of mosquitofish and is certainly related to their particular life-history strategy, combining an early maturation and short lifespan with the physiological costs of reproduction and over-wintering. Our findings probably apply to other small, short-lived species with similar life history.

Keywords: age, body size, *Gambusia holbrooki*, mosquitofish, Poeciliidae, reproductive investment

Introduction

Trade-offs between vital functions, such as growth, reproduction and survival have been the subject of many experimental, theoretical and empirical studies aiming to evaluate the ecological performance of organisms. A resultant basic principle is that reproduction has a cost in terms of future survival and breeding success leading to a trade-off between present progeny and the expectation of future reproduction. Early theoretical studies predict that reproductive effort should increase with age, as the expectation of subsequent reproduction decreases (Pianka & Parker, 1975; Charlesworth & Leon, 1976). Evidence of this age-related increase in reproductive investment has been reported in a wide range of animals, at least for iteroparous organisms and before senescence (Forslund & Pärt, 1995; Weladji *et al.*, 2002; Broussard *et al.*, 2003).

While age-related changes occur in all organisms (Roff, 1992), much of this framework is based on taxa with determinate growth, such as mammals or birds, in which the occurrence of a fixed maximum size (Peters, 1983) has certainly motivated studies on changes in reproductive values with ageing (e.g. Crocker *et al.*, 2001; Reid *et al.*, 2004). In the case of organisms that continue to grow throughout their lives, much attention has been focused on size-dependence of reproductive values (e.g. Schultz, Clifton & Warner, 1991; Fox & Crivelli, 1998; Bonnet *et al.*, 2000; Lüddecke, 2002; Du, Ji & Shine, 2005). Indeed, increased progeny size, weight, number or other reproductive descriptors of older individuals need not to denote a higher reproductive effort. Evidence of absolute increases in reproductive performance with age does not constitute evidence of increased investment, since older individuals may have higher rates of energy acquisition because of their increased size. For instance, age and body size in fish are closely related

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(Pitcher & Hart, 1982) and when the effect of size is removed, the effect of age on fecundity may be small or inconsistent (Wootton, 1979).

The relative weight of gonads (i.e. ovary weight) has been widely used as an index of reproductive effort in fish (Roff, 1992), and is mostly relevant for species without parental care (e.g. see Gillooly & Baylis, 1999). Within fish species there is an allometric relationship linking gonad weight to body weight (e.g. Roff, 1992; Gunderson, 1997; Somarakis *et al.*, 2004), such that a positive linear relationship is observed when these variables are log-transformed which by implication reduces the influence of age on reproductive effort. However, age-specific variations in traits related to reproduction have been observed in long-lived fish species independent of their size (Van Eenennaam & Doroshov, 1998), or have been indirectly related to reproductive effort by age-specific survivorship (Hutchings, 1993; Bertschy & Fox, 1999).

Small, short-lived livebearing fish such as *Gambusia* provide unusual opportunities to test for changes in reproductive effort with age. These species produce litters of live born young approximately every 3 weeks from late spring throughout late summer (Krumholz, 1948; Reznick & Braun, 1987). In addition, early-born young can reproduce in the season of their birth while those born at the end of the reproductive season must over-winter before reproducing (Krumholz, 1948), the latter spending part of the fat reserves built up during late summer (Reznick & Braun, 1987). Life-history studies on mosquitofish [*Gambusia affinis* (Baird & Girard, 1853) and *G. holbrooki* (Girard, 1859)] have provided evidence suggesting a differential reproductive advantage (i.e. fitness) between successive clutches (Reznick *et al.*, 2006) or different reproductive tactics (i.e. differences in size and numbers of offspring) in two successive cohorts (Fernández-Delgado, 1989; Fernández-Delgado & Rossomanno, 1997). Here, we report on reproductive effort in *G. holbrooki* sampled simultaneously but of different ages. Following the above lines of evidence, we expected a decline in the rate of increase in female gonad weight in relation to body weight (or size) from individuals of successive years. This prediction opposes the common allometric relationship linking gonad weight to body weight (e.g. Roff, 1992; Gunderson, 1997; Somarakis *et al.*, 2004). We therefore aimed to detect any nonlinear relationship between log-transformed gonad weight and body size, with such nonlinearity coinciding with age changes and therefore indicating a size-independent age effect on reproductive effort.

Methods

Sampling and data

A total of 18 populations of *G. holbrooki* was sampled from the downstream sections and river mouths of 11 Mediterranean drainage basins in eastern Spain (Almanzora, Ebre, Fluvià, Millars, Muga, Ter, Tordera, Segura and Xúquer drainages) and southern France (Orb and Bourdigou drainages) from late June (27th) to early July (6th) 2005, to get a maximum number of individuals in similar reproductive condition (Reznick *et al.*, 2006) from each age class. To attain a sufficient number of mature females, similar localities (i.e. presence of reed beds, 1.5 m maximal depth, low water velocity and fine depositional substrata) were sampled using dip nets and complemented with other sampling methods (fyke nets, minnow traps and electrofishing). Specimens were preserved *in situ* in 96% ethanol and brought to laboratory, measured with digital callipers (standard length to the nearest 0.01 mm) and weighed (weights recorded were of the fish after evisceration – removal of all organs – and gonad weight, to the nearest 0.1 mg). Gonad weight can be considered an accurate proxy of reproductive effort since there is neither parental care nor is there any site preparation for spawning and the species is not territorial (Krumholz, 1948). Sex was determined from the morphology of the anal fin (Turner, 1941) or, when not possible, by direct observation of the gonads. Six to eight scales from the left side of the body between the lateral line and the dorsal fin were removed for age estimation under a dissecting microscope. Age was determined by scale reading of 326 females complemented by length-frequency distribution analysis, yielding 769 aged mature females. A potentially confounding effect of river system with age class was rejected because no bias in age class representation was found between rivers, with all populations having the greatest abundances of young of the year (0+) followed by 1-year old (1+) and 2-year old fish (2+) (Friedman rank test, $P > 0.05$).

Statistical analyses

The hypothesis to test is a nonlinear log-scale relationship between gonad weight and body size, with such nonlinearity matching age changes. Variation in gonad weight as a function of age class and the interaction between age and size were tested with an analysis of covariance (ANCOVA) using fish size as the covariate (García-Berthou & Moreno-Amich, 1993). This first test assessed the influence of age on the rate of increase of gonad weight with fish size and was complemented with the following analyses aiming to confirm that the nonlinear pattern is actually a consequence of the age effect.

To take into account the nonlinearities suggested by the data, a generalized additive model (GAM, Hastie & Tibshirani, 1990) of gonad weight against body size was performed. The significance of the improved fit was assessed by a likelihood ratio test based on the difference (D) between the deviances of the linear and nonlinear models. Under the null hypothesis that the nonlinear approach does not significantly increase the fit to the data compared to the linear regression, D is distributed as a χ^2. The same procedure was applied for each age class separately to confirm the implicit assumption that, within age classes, gonad weight increases linearly with fish size.

To further assess if the nonlinear pattern matched the successive age changes, we applied a parametric linear additive model (Hastie & Tibshirani, 1990) with internal breakpoints in the range of the predictor (i.e. body size). These breakpoints were as many as n-1 age classes, presumably two, since *G. holbrooki* has a maximal lifespan of 2 years (i.e. $n = 3$ age classes) in the populations studied and elsewhere (Krumholz, 1948). They were obtained by a recursive procedure with a varying breakpoint ranging from the smallest to the largest observed size (the spacing between successive breakpoint values in the sequence was 0.001 log-body size), and then selecting the model that minimizes the residual variance. The second breakpoint was achieved by the same procedure but starting the sequence of successive breakpoint values at the first one. The significance of the fitted models was assessed by the change in residual variance between the nested models (linear, one breakpoint and two breakpoints models). The observed breakpoint values were finally compared to the expected points, in the body size range, where dominance in abundances switched between two successive age classes. The difference between these parameters was evaluated by the residual standard errors of the recursively fitted models which provided the expected distribution under the null hypothesis of no difference between the observed and expected residual values, against which the statistical significance of the observed difference was assessed. Statistical analyses were performed using the S-PLUS 2000 software package (Mathsoft, Inc., Seattle, WA, U.S.A.).

Results

Three age classes were found (0+, 1+ and 2+ females) and the ANCOVA testing for an effect of age on gonad weight, after factoring out body size, revealed a significant influence of age ($F_{2,763} = 125.7$, $P < 0.0001$) and a significant interaction between age and body size ($F_{2,763} = 131.7$, $P < 0.0001$). The interaction term suggests significant differences in slopes of the positive gonad weight–body size relationships between age classes (Fig. 1a). However, these differences between slopes were only detected when comparing ages 0+/1+ ($F_{1,744} = 243.6$, $P < 0.0001$) and 0+/2+ ($F_{1,523} = 16$, $P < 0.0001$), while no significant difference was found when comparing ages 1+/2+ ($F_{1,259} = 0.95$, $P = 0.33$), although an age effect is still present between these older classes ($F_{1,259} = 9.8$, $P = 0.002$). The allometric relationship between eviscerated weight and body size (Fig. 1b) shows an unambiguous linear pattern and therefore supports the single nonlinear effect of fish size on gonad weight, excluding a potential effect of somatic mass.

The smoothed curve from the fitted GAM (Fig. 2a) shows that the rate of the positive trend declines at intermediate sizes. This nonlinear pattern performs significantly better than fitting a linear relationship between the log-transformed gonad weight and body size ($P < 0.0001$). Within each of the three age classes, the nonlinear GAM approach did not produce a significant change in deviance in comparison to a linear model ($P > 0.05$). This finding implicitly supports the hypothesis of nonlinearity in the gonad weight–body size relationship as a consequence of ageing (see below for a further test).

Since three age classes were found, the model fitted by the linear additive approach (Fig. 2b) displayed two breakpoints. Adding the first breakpoint significantly improved the fit compared to a linear

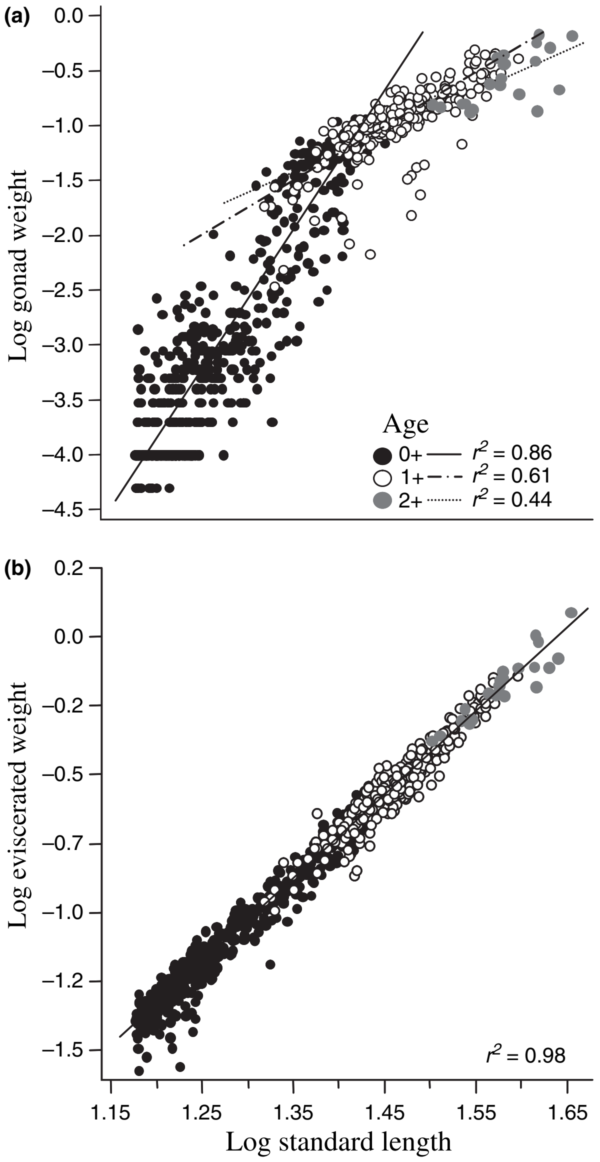

Fig. 1 (a) Gonad and (b) eviscerated weights as a function of standard length for the mosquitofish studied. Linear regressions for each age class are displayed for gonad weight, and a single linear relationship is presented for eviscerated weight.

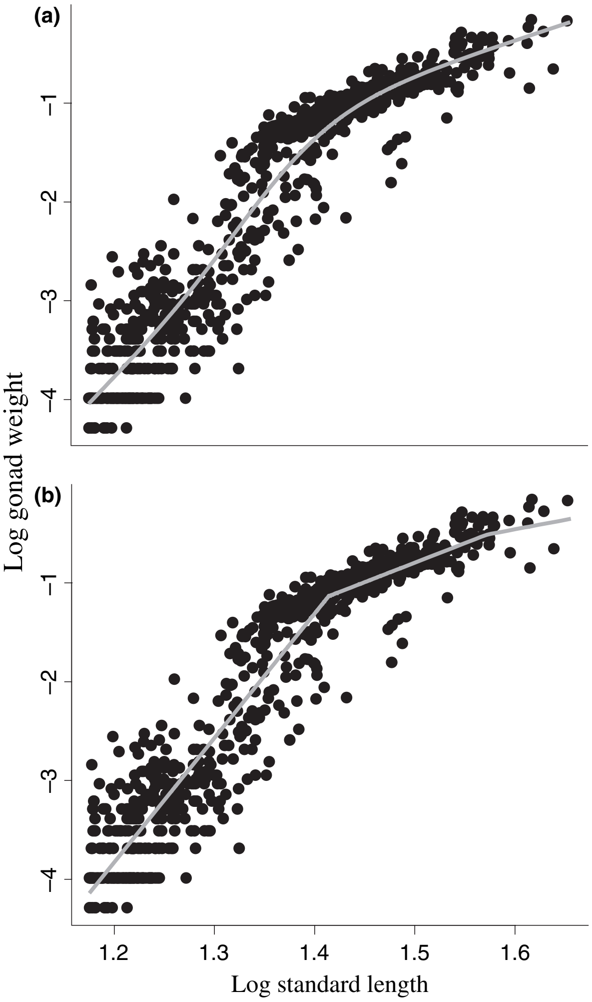

Fig. 2 Gonad weight as a function of mosquitofish standard length with additive model fits: (a) the smoothed curve of the generalized additive model and (b) the linear additive model with two breakpoints corresponding to age changes.

regression ($F_{1,766}$ = 394.7, $P < 0.0001$), whereas adding the second did not ($F_{1,765}$ = 0.60, P = 0.44), compared to the former. The best fit (i.e. least residual variance) was achieved with breakpoint values at 1.414 and 1.572 (log-standard length in mm) that clearly matched to the points where dominance in numbers switched between two successive age classes, 1.425 and 1.570, respectively, between 0+/1+ and 1+/2+. These expected (i.e. the points where dominance switches between age classes) and observed values (i.e. the points obtained for the best fitted model) did not differ significantly ($P > 0.95$), again supporting the nonlinear pattern as a consequence of age changes.

Discussion

All the above lines of evidence suggest a nonlinear relationship between gonad weight and fish size clearly affected by age changes, with a declining rate of increase of gonad mass as a function of body size, revealing a higher reproductive effort for younger individuals relative to size. Our results support previous studies providing evidence of differential reproductive output with ageing in female mosquitofish (Fernández-Delgado & Rossomanno, 1997;

Reznick *et al.*, 2006), and go a step further by pointing out a nonlinear relationship of log-transformed variables as a consequence of this age effect. This finding contrasts with the linear relationships commonly observed in longer-lived fish species. A similar pattern, although not explicitly tested, was found in female *Micrometrus minimus* (Gibbons, 1854), a marine fish species presenting a similar short-lived viviparous life history (Schultz *et al.*, 1991) as mosquitofish, suggesting that the observed pattern is to be related to their particular biological traits and life-history strategy.

A basic principle of life-history theory is that reduced adult survival will select for earlier maturation and increased reproductive effort (Stearns, 1989; Roff, 1992). To achieve sustainable fitness levels, species with a short lifespan must direct important amounts of resources towards reproduction, reducing those available for growth and survival. After the first month in the year of their birth, mature females of *G. holbrooki* do not grow in size (Vargas & De Sostoa, 1996; Fernández-Delgado & Rossomanno, 1997), diverting all energy to the production of offspring; this contrasted with the following year, when a size increase was observed. Extreme cases in fish taxa are found among annual species that reproduce and die within the same year, death being probably a direct consequence of the physiological cost of reproduction (e.g. Crivelli & Britton, 1987; Poizat, Rosecchi & Crivelli, 1999). In the present case, the over-winter survival of females and the associated energy depletion (Reznick & Braun, 1987) are probable causes of the observed pattern. Winter is a stressful period for fishes, as reflected by substantial over-winter declines in fat reserves (e.g. Schultz & Conover, 1997; Eckmann, 2004), mosquitofish being no exception. The species stops reproducing in the late summer, before the end of warm weather, stores fat and then uses these reserves to survive the winter and initiate reproduction the following spring (Reznick & Braun, 1987). The annual lipid cycle of *G. holbrooki* is closely linked to its reproductive timing (Reznick & Braun, 1987; Meffe & Snelson, 1993), and the very low fat storage found in spring (all individuals being 1+ and some 2+) is probably responsible for the observed pattern.

As *G. holbrooki* reproduces frequently (every 3 or 4 weeks), the cost of previous reproductions could partly account for the nonlinear relationship observed between size and reproductive effort. If the energetic cost of previous reproductions was acting, however, we would observe separable curvilinear relationships between gonad weight and size for each age class, because larger individuals have undergone more reproductive events. Our results show linear relationships, clearly rejecting this possibility.

As part of the theory of senescence (Kirkwood, 2005), a similar evolutionary trade-off is expected between traits that enhance reproduction early in life and the investment in maintenance and reproduction later in life (e.g. Weladji *et al.*, 2002; Broussard *et al.*, 2003), as a consequence of physiological deterioration of individuals at advancing ages. Senescence could then account for our results since it addresses biological processes underlying the decline in age-specific fitness components, as an organism ages (e.g. a decline of reproductive effort with age). However, as senescence and optimal life histories can independently produce the same phenotypic patterns in reproductive effort and survival (Blarer, Doebeli & Stearns, 1995), it is impossible to discern between these two theories from our results. Further studies are necessary to understand the interaction between intrinsic (i.e. senescence) and extrinsic (i.e. overwintering) mortality factors driving reproductive investment in mosquitofish (see a recent example with guppies by Reznick *et al.*, 2004).

The observed age effect on reproductive effort has considerable implications in the study of mosquitofish life history and may be of general concern for other small, short-lived species such as cyprinodontiform fishes, where over-wintering represents almost half of their lives, implying stronger consequences on their evolutionary ecology than for longer-lived species. Our findings suggest that seasonal or spatial variations in age structure should be addressed when assessing reproductive effort and related traits, and in cases where age is unknown, potential age effects might be detected with nonlinear or discontinuous relationships. Furthermore, because age-dependent patterns of reproductive performance can influence population dynamics (e.g. Reed *et al.*, 2003), understanding how reproduction varies with age is an important area for development in life-history theory and may be of critical interest when considering invasive species such as mosquitofish.

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