Empirical evidence of a trade-off between reproductive effort and expectation of future reproduction in female three-spined sticklebacks

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Optimal life-history models generally predict that the reproductive effort of iteroparous organisms may increase with age, as their expectation of future reproduction decreases. The population of three-spined sticklebacks (*Gasterosteus aculeatus*) in the Camargue (Rhône River Delta, France) is annual, all adults dying after their first breeding season. As the three-spined stickleback is a multiple spawner, we tested the hypothesis that reproductive effort may increase during the breeding season on field data. From 1987 to 1998, 653 female sticklebacks were collected in the field during the breeding seasons. The body size, body weight and weights of the liver, gonads and carcass were measured for these individuals. Only gravid females with mature eggs (176 fish) were included in the analysis. Considering the female three-spined stickleback as a capital breeder, the energetic resources available for allocation between soma and gonads were estimated by its body weight. Somatic condition decreased during the breeding season and reproductive effort (gonad weight relative to body weight) increased. These patterns did not vary significantly between years. These observed variations in reproductive effort during the breeding season can be interpreted as empirical evidence of a trade-off between reproductive effort and expectation of future reproduction.

Keywords: life history; resource allocation; multiple spawning; fish; *Gasterosteus aculeatus*

1. INTRODUCTION

One of the basic principles of life-history theory is that reproduction has a cost in terms of subsequent survival and breeding success. This cost leads to a trade-off between reproductive effort at any given time and expectation of future reproduction (Fisher 1930; Williams 1966). Optimal models generally predict that reproductive effort may increase with age, as the expectation of future reproduction decreases (Williams 1966; Gadgil & Bossert 1970; Schaffer 1974; Pianka & Parker 1975; Charlesworth & Leon 1976). Indications of an increase in reproductive effort with age have been given in various animals (Clutton-Brock 1991, p. 178; Roff 1992, p. 267). However, there are several methodological problems in providing evidence of this prediction from real data (Pianka & Parker 1975; Hirschfield 1980; Clutton-Brock 1984; Reznick 1985; Van Noordwijk & De Jong 1986; Pease & Bull 1988; Schultz & Warner 1991). One of the main problems is correctly taking into account the differences in energy resources between individuals in order to compare their resource allocation patterns. The 'capital', i.e. the individual's body reserves, is usually satisfactorily estimated by body weight, but, in contrast, the 'income', i.e. the energy input from food during the breeding season, is logistically much more difficult to quantify

individually (Van Noordwijk & De Jong 1986; Doughty & Shine 1997). Drent & Daan (1980) and then Stearns (1989) and Jönsson (1997) distinguished between 'income breeders' and 'capital breeders' as two extremes along a continuum, ranging from organisms in which the energy allocated to reproduction is derived exclusively from food consumed during the breeding season and organisms whose energy allocation to reproduction is derived from reserves built up beforehand. As the main methodological difficulty is quantifying income, Doughty & Shine (1997) considered that capital breeders offer good opportunities for analysing resource allocation, as the capital is a good approximation of the energy resources that such organisms possess.

There have been many experimental studies on the mechanisms of resource allocation in female three-spined sticklebacks (*Gasterosteus aculeatus* L.), mainly conducted by R. J. Wootton and his colleagues (Wootton 1973, 1977, 1994; Allen & Wootton 1982; Fletcher & Wootton 1995). In this fish species, it would appear that food intake (income) influences the number of spawnings during the breeding season, the interval between them and the somatic condition of females, but has no direct effect on the characteristics of each spawning. The energy allocated to a particular spawning can come either from food or from depleting body reserves and this last physiological way allows the energy allocated to a spawning to be independent of food resources. In fact, the energy requirement for egg production is so high that depletion of body

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reserves is usually observed. Therefore, in accordance with Doughty & Shine (1997), we consider that the three-spined stickleback offers good opportunities for analysing the investment in each spawning since it can be considered as a capital breeder at the scale of a single spawning.

The population of three-spined sticklebacks in the Camargue, at the southern limit of the species' distribution range, differs from other three-spined stickleback populations in its short life span, its brief and early breeding season, its high investment in reproduction, its high fecundity in relation to size and its small eggs (Crivelli & Britton 1987; Baker 1994; J. A. Baker, personal communication). At the end of the breeding season, all the adults die. According to Crivelli & Britton (1987), their death is probably a direct consequence of the physiological cost of reproduction, since (i) environmental conditions are still favourable for survival and (ii) adults have extremely low somatic conditions. Camargue sticklebacks therefore occupy an extreme position in the trade-off between reproductive effort and expectation for future reproduction, investing very heavily in reproduction at the age of one year and not surviving beyond this.

The three-spined stickleback is a multiple spawner, i.e. a female can spawn several times during the same breeding season at a few days interval (Wootton 1984) and multiple spawning can be considered as within-season iteroparity (Pianka & Parker 1975; Kirkendall & Stenseth 1985). Then, the application of the theory of age change of reproductive effort leads to the prediction that reproductive effort should increase during the breeding season. The aim of this paper is to test this prediction by analysing variations in reproductive effort and somatic condition during the breeding season with female three-spined sticklebacks collected from 1987 to 1998 in the Camargue natural population.

2. MATERIAL AND METHODS

(a) *Study area*

The Camargue is a wetland complex situated in the Rhône River Delta on the Mediterranean coast of France. Sticklebacks occur here in three types of water body: (i) shallow freshwater or oligohaline ($< 5 \text{ g l}^{-1}$ salinity) marshes and ponds with abundant vegetation which dry out in summer, (ii) drainage canals that are permanently full of water and which flow into mesohaline lagoons and (iii) mesohaline lagoons ($5\text{--}30 \text{ g l}^{-1}$ salinity) most of which remain flooded throughout the year. The drainage canals are connected to the mesohaline lagoons throughout the year.

The present study deals with the three-spined sticklebacks that migrate into the catchment of the Fumemorte drainage canal to breed, including the main canal, its branches and the connected marshes.

(b) *Sticklebacks' life cycle in the Camargue*

Three-spined sticklebacks occurring in the Camargue belong to the low-plated *leiurus* type (Crivelli & Britton 1987). Their life cycle is anadromous, reproducing in freshwater canals or marshes with the young migrating back to brackish lagoons to pass the summer and autumn (Crivelli & Britton 1987; Pont *et al.* 1991; Poizat & Crivelli 1997). The population is strictly annual and the breeding season lasts for *ca.* 50 days, occurring around March (Crivelli & Britton 1987).

(c) *Fish data*

Fish were captured in the Fumemorte canal and in other canals of its drainage basin using fyke nets of 5 mm mesh size and traps of 1 mm mesh size during at least one week per month in January–April of 1987–1998. Only mature females containing yolky eggs, i.e. stage 5 of Nikolski (1963), were included in the analyses, so as to eliminate the stage of advancement of oogenesis as a source of variation in reproductive traits between individuals. By choosing the most advanced stage of egg development, the best description of investment in reproduction and reproductive output was obtained. Females having already started to spawn their present clutch were not included in the analyses because their gonad weight would have been underestimated.

The fish were preserved in 10% formol before being measured and dissected. Their total body length was measured to the nearest millimetre. Carcass dry weight was measured after drying at 70°C for 48 h.

(d) *Data analysis*

The energy available to each fish was estimated from its total body weight. This energy resource was divided into energy allocated to reproduction and energy allocated to the soma. The liver was considered as occupying an intermediate position between the soma and the gonads because it is involved in providing the energy that is invested in reproduction, since it synthesizes yolk precursors (Wootton 1984) and acts as a buffer between the carcass and the gonads, allowing the latter to grow independently of food intake (Allen & Wootton 1982; Chellappa *et al.* 1989). Because of this physiological position of the liver, we used the carcass weight without the liver and other viscera as a measure of somatic weight, instead of total weight minus gonad weight.

The relationship between the weight of each body component and body length was fitted to a power function, using a linear regression model on log-transformed variables (log–log regression). As there was a significant relation to length, the length effect was eliminated by taking the residuals of the log–log regression as a relative measure of body component weight.

The somatic condition was measured by the relative carcass weight, i.e. the residuals of the log–log linear regression between carcass weight and body length. The energy allocated to reproduction was measured by the weight of the gonads. To compare the reproductive effort between individuals, differences between individuals in total energy resources have to be eliminated. To do this, reproductive effort was measured by the residuals of the log–log linear regression between gonad weight and total weight.

Temporal changes in each biological variable were analysed by linear regressions between the variable and the date (day number counting from 1 January of each year). Between-year variations of within-year patterns were tested by comparing the slopes of the regressions for each year using an *F*-test (Wilkinson 1992). Only years with more than five measured individuals were included in these tests.

3. RESULTS

From 1987 to 1998, 653 adult female three-spined sticklebacks were captured during the breeding season and dissected. Of these, 176 fish complied with the selection criteria that were imposed. All the body component weights showed a strong positive correlation with female

Table 1. Range of observed values and coefficients of the log–log linear regression between biological variables and body length (mm) of female sticklebacks which were ready to spawn

trait	minimum	maximum	slope	intercept	<i>n</i>	<i>r</i>	<i>p</i>
body length (mm)	32.000	64.000	—	—	—	—	—
body weight (wet, g)	0.487	3.781	—	—	—	—	—
carcass weight (dry, g)	0.061	0.583	3.122	−13.674	176	0.924	< 0.001
liver weight (wet, g)	0.019	0.301	3.389	−15.568	174	0.800	< 0.001
gonad weight (wet, g)	0.112	1.301	3.256	−13.365	175	0.857	< 0.001

Table 2. Coefficients of the linear regression of traits and date within the breeding season (number of days since 1 January)

(Relative carcass weight (RCW), residuals of carcass weight to body length; relative liver weight (RLW), residuals of liver weight to body length; relative gonad weight (RGW), residuals of gonad weight to body length; reproductive effort (RE), residuals of gonad weight to body weight.)

trait	slope	intercept	<i>n</i>	<i>r</i>	<i>p</i>
body length	−0.0303	49.615	176	−0.097	0.200
body weight	−0.0038	1.919	176	−0.107	0.157
RCW	−0.0045	0.322	176	−0.532	< 0.001
RLW	−0.0005	0.035	174	−0.030	0.698
RGW	0.0014	−0.098	175	0.108	0.155
RE	0.0016	−0.113	175	0.201	0.008

body length (table 1). Therefore, the residuals of the log–log regressions with size were used as relative measurements. The relative liver weight was positively correlated with the relative carcass weight ($n=174$, $r=0.198$ and $p=0.009$). The relative gonad weight was positively correlated with the relative liver weight ($n=173$, $r=0.457$ and $p<0.001$). The relative gonad weight was positively correlated with the relative carcass weight ($n=175$, $r=0.162$ and $p=0.033$).

Body length, body weight, relative liver weight and relative gonad weight did not have a significant linear relationship with the date (table 2; figure 1). The relative carcass weight decreased very clearly during the breeding season (table 2; figure 1) and the slopes did not differ significantly between years ($F_{9,150}=1.043$ and $p=0.409$).

Multiple regression of the relative gonad weight with the relative carcass weight and date (table 3) showed that the relative gonad weight was positively correlated with both of these variables. The strong negative correlation between relative carcass weight with date (table 2) explains why no significant simple correlation was found between the relative gonad weight and date. This result suggests that, if the relative carcass weight was held constant, the relative gonad weight may increase during the breeding season. To explore this hypothesis empirically, we separately assessed a regression of the relative gonad weight with date on individuals with high relative carcass weight (positive residuals of carcass weight to body length) and on individuals with low relative carcass weight (negative residuals of carcass weight to body length) (figure 2). Individuals with a high relative carcass weight were found to show an increase in their relative gonad weight during the breeding season ($n=92$, $r=0.315$ and $p=0.002$). This tendency did not vary significantly between years ($F_{7,62}=0.723$ and $p=0.653$). Individuals with a low relative carcass weight did not

show any linear tendency ($n=83$, $r=0.054$ and $p=0.626$) and there was no interaction between the year and the date within the year ($F_{6,62}=1.754$ and $p=0.123$). Multiple regression of the relative liver weight with relative carcass weight and date (table 3) did not detect any significant partial correlation with the date.

Reproductive effort was calculated from the residuals of the linear regression between log gonad weight and log total body weight (slope=1.123, intercept=−1.293, $n=175$, $r=0.947$ and $p<0.001$). Reproductive effort increased during the breeding season (table 2; figure 1). Comparison of the slopes between years showed no significant differences ($F_{9,149}=0.808$ and $p=0.610$).

4. DISCUSSION

In this study population of three-spined sticklebacks, we observed a decrease in somatic condition and an increase in reproductive effort during the breeding season in females which were ready to spawn. In an annual population, the date within the breeding season can be considered as indicating the expectation of future spawning (Sargent & Gross 1985; Ukegbu & Huntingford 1988; Candolin 1998). Therefore, our results suggest a trade-off between reproductive effort and expectation of future reproduction.

During the breeding season, the body length and body weight of females which were ready to spawn did not show any linear tendency. When considered separately, the gonad weight (relative to body length) of high somatic condition individuals showed an increase during the breeding season, whereas no tendency was detected in low somatic condition individuals. This suggests that there is a potential tendency for increase in gonad weight during the breeding season, but which is realized only when somatic reserves are sufficiently high to allow it.

Figure 1. (a-d) Variation in biological variables of ready-to-spawn female sticklebacks with date within the breeding season. The regression line is given when correlation is significant (see table 2). Relative carcass weight (RCW), relative liver weight (RLW), relative gonad weight (RGW) and reproductive effort (RE) (see the text and table 2 for definitions of these variables).

Figure 2. Variation in relative gonad weight (RGW) of ready-to-spawn female sticklebacks with date within the breeding season for (a) high somatic condition individuals ($RCW > 0$) and (b) low somatic condition individuals ($RCW < 0$). The regression line is given when correlation is significant (see the text).

The intermediate physiological position of the liver between the soma and gonads (Allen & Wootton 1982; Wootton 1984; Chellappa *et al.* 1989) explains why its relative weight was positively correlated with both gonad weight and carcass weight and why the liver weight did not show any tendency to either increase or decrease during the breeding season.

In an annual population of three-spined sticklebacks in Scotland, Chellappa *et al.* (1989) observed that males that did not breed survived beyond the breeding season and even accumulated reserves in their bodies. This strongly suggests that the mortality of males that breed is caused by their investment in reproduction. Similarly, in our study population, the sharp decrease in female somatic condition seems to be (i) a consequence of reproductive effort and (ii) responsible for adult post-breeding mortality. However, studies at the individual level are needed to prove these causations.

In the above-mentioned Scottish population, Chellappa *et al.* (1989) found that males that did not breed did finally die in winter, probably because of the decline in food resources. Similarly, in the Camargue, it seems very probable that adults that did not die immediately after breeding would be unable to withstand the high summer water temperatures (mean daily water temperature $> 25^{\circ}\text{C}$; Poizat & Crivelli 1997) and would die before

Table 3. Multiple regression coefficient of relative gonad weight (RGW) and relative liver weight (RLW) with relative carcass weight (RCW) and date within the breeding season

dependent variables	n	independent variables	coefficient	p
RGW	175	constant	-0.246	0.003
		RCW	0.461	< 0.001
		date	0.003	0.002
RLW	174	constant	-0.125	0.243
		RCW	0.501	0.004
		date	0.002	0.229

reaching the next breeding season. The disposable soma theory (Kirkwood & Rose 1991) provides a physiological explanation for the evolution of a decrease in somatic condition with age. If environmental mortality (from extrinsic factors such as predation, food shortage or abiotic factors) is high, then, as the energy invested in somatic maintenance has a high probability of being wasted, the optimal investment in the soma is lower than that needed to maintain a constant somatic condition, thereby allowing greater reproductive investment. This theory applies very well to the three-spined stickleback, in which energy allocated to the soma is of low priority since the energy allocated to reproduction can come from depletion of the soma (Wootton 1994).

Attempts to provide evidence of age changes in reproductive effort as a consequence of a trade-off between reproductive effort and expectation of future reproduction have been numerous (reviewed by Roff 1992, p. 267). However, few are convincing because other factors may be responsible for the variations observed (Clutton-Brock 1991, p. 178). Older individuals may differ from younger individuals in several morphological, physiological, behavioural and ecological traits which may interfere with the possible consequences of a trade-off between present and future reproduction (Clutton-Brock 1984).

In the present study, differences in individual energy resources could be controlled because the female three-spined stickleback is considered as a capital breeder in light of experimental studies on resource allocation (Wootton 1994; Fletcher & Wootton 1995). Therefore, individual energy resources were correctly estimated by body weight (Doughty & Shine 1997). To measure reproductive effort independently of an individual's energy resources, we used residuals of an allometric regression between gonad weight and body weight. In contrast, the classical ratio between clutch weight (or gonad weight) and body weight does not constitute a measure of reproductive effort independent of body weight, because a given reproductive effort does not vary isometrically with body weight. Allometric relationships (power functions) are much more suitable (Calder 1984).

Changes in reproductive effort could result from causes other than evolutionary ones. For instance, environmental changes during the breeding season may have a proximate influence on resource allocation patterns (Pianka & Parker 1975; Clutton-Brock 1984). This could not be controlled in the present field study. However, experimental studies have shown that food regime does not

influence reproductive effort per clutch in the three-spined stickleback (Wootton 1994; Fletcher & Wootton 1995). Unfortunately, no similar experimental studies are available on the effect of temperature on sticklebacks' resource allocation patterns.

The present study opens up two main perspectives for further research. First, patterns that have been observed at the population level should be confirmed and detailed at the individual level using an experimental approach (e.g. Fox & Crivelli 1998). Second, the great diversity in age at maturity and longevity among three-spined stickleback populations (Baker 1994) offers good opportunities for testing the influence of adult survival between breeding seasons on changes in reproductive effort during the breeding season.

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