REPRODUCTIVE SUCCESS OF SMALLMOUTH BASS ESTIMATED AND EVALUATED FROM FAMILY-SPECIFIC DNA FINGERPRINTS

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Abstract. This paper presents the results of the first attempt to estimate and identify factors influencing individual reproductive success in a fish species, under natural conditions, after the progeny have dispersed from their site of origin. Using family-specific DNA fingerprints, the reproductive success of male smallmouth bass in Lake Opeongo, Ontario was estimated and evaluated at the point when their offspring were fall young-of-the-year (YOY). For the 1992 spring spawning season, we generated family-specific fingerprints using DNA of fry captured from 116 nests in Jones Bay. In the fall of the same year, 283 YOY were captured by electroshocking along the shoreline of the study area. Using DNA obtained from fin tissue, a fingerprint was generated for each fall YOY and compared to the family fingerprints to determine the family of origin. Males were considered successful if at least one of their offspring survived to the fall YOY stage. Although 27.7% of all males who acquired eggs \( (N = 57) \) had at least one offspring surviving to the fall YOY stage, only 5.4% of all spawning males \( (N = 11) \) produced 54.7% of the total number of fall YOY captured. If recruitment frequently depends on the success of such a small number of males, then population abundance will be extremely vulnerable to adverse natural and anthropogenic impacts. Because natural resource managers cannot differentiate these few successful males a priori, they cannot differentially protect them from potentially adverse human activities, such as opening the fishing season on a preset date that may fall before the end of brood-guarding in a given year.

The evaluation of variables that might influence differences in male reproductive success included male length and age, spawning date, number of eggs and fry within a nest, nest depth, and dominant substrate type both within and surrounding the nest. Logistic regression analysis indicated that none of these variables were significant predictors of male reproductive success \( (P > 0.05) \). Additionally, linear regression analysis suggested the lack of a relationship between the number of fall YOY produced per male and these variables. However, a goodness-of-fit test to the Poisson distribution indicated that the number of YOY produced per male does not occur at random \( (P < 0.001) \), suggesting that reproductive success was influenced by factors not measured in this study.

Key words: DNA fingerprinting; family size; genetic marks; Lake Opeongo, Ontario; reproductive success; Micropterus dolomieu; smallmouth bass; young-of-the-year.

INTRODUCTION

Field measurement of individual reproductive success beyond early life stages is extremely difficult or impossible for many species due to the inability to identify and subsequently monitor the survival of offspring from a particular mating. For fishes, these difficulties are accentuated by the aquatic environment, and the reproductive strategies of many species make it impossible to study reproductive success. Many fishes reproduce by the external fertilization of gametes that are dispersed in the aquatic environment, so it is difficult to determine parentage. Therefore, parentage and reproductive success can be assessed most easily in fish species that construct nests or provide parental care. For example, factors influencing individual reproductive success have been evaluated in field studies for members of the following fish families: Centrarchidae (Goff 1986, Noltie and Keenleyside 1986, Dupuis and Keenleyside 1988, Wiegmann et al. 1992, Hunt and Annett 1993, Lukas and Orth 1993); Chaenopsidae (Hastings 1992); Cichlidae (Fitzgerald and Keenleyside 1978, Mrowka 1987, Raadik et al. 1990); Cottidae (Goto 1993); Gasterosteidae (Kynard 1978, Goldschmidt and Bakker 1990); Percidae (Grant and Colgan 1983); and Pomacentridae (Schmale 1981, Thresher 1983).

In all of the studies cited above, the survival of offspring was monitored through perceived critical life stages, either embryos or fry, and this served as an operational measure of reproductive success. Evaluation of reproductive success has been restricted to these early brood-guarding stages, when survival is relatively high, because offspring cannot be monitored once they disperse and mix with offspring from other families.
For this reason, prior studies have been limited in their ability to assess the factors influencing individual reproductive success beyond the brood-guarding stages. The primary reason offspring have not been monitored after dispersal is that there are currently no techniques available to apply individual marks to larval fish due to their small size. One approach to overcoming this problem is to identify genetic marks, such as those generated by DNA fingerprinting (Jeffreys et al. 1985), that are capable of distinguishing individual fish (Fields et al. 1989, Castelli et al. 1990, Whitmore et al. 1990) and possibly family members.

Gross et al. (1994) used DNA fingerprinting to generate family-specific marks for smallmouth bass (Micropterus dolomieu). These family-specific marks provided the first opportunity to assess individual reproductive success for a fish species to any life stage of its offspring in the wild, and to elucidate the environmental and biological factors controlling success.

The spawning behavior of smallmouth bass has permitted the evaluation of factors influencing their reproductive success, though only up to the embryonic (Neves 1975, Winemiller and Taylor 1982) or swim-up fry stages of their offspring (Goff 1986, Raffetto et al. 1990, Reynolds 1990, Ridgway and Friesen 1992, Wiegmann et al. 1992, Lukas and Orth 1995). Smallmouth bass spawn in late spring and early summer at water temperatures ranging from 12.8–20.0°C (Scott and Crossman 1973). The male builds a saucer-shaped nest, usually in gravel or rocky substrate, by fanning out an area with his fins until he has cleared an area ø 0.5 m in diameter (Breder and Rosen 1966). After spawning, the male guards his offspring for up to several weeks after the eggs have hatched. Upon hatching, the fry initially remain in a cluster, and can be collected from their nest to obtain tissue samples for generating family-specific DNA fingerprints.

This paper describes the application of DNA fingerprinting to assess male reproductive success in the natural population of smallmouth bass in Lake Opeongo, Ontario. Family-specific marks were generated from the offspring of each nest in Jones Bay, Lake Opeongo during the 1992 spring spawning season. We determined the survival rate of the offspring from each male by capturing and fingerprinting 1992 fall young-of-the-year (YOY), and matching their fingerprints to family DNA fingerprints to determine their families of origin. As a first step toward the identification of ecological factors affecting reproductive success to later life stages, we also present analyses of variables that previous researchers found had influenced male reproductive success to embryonic or fry stages.

METHODS

Study site and sample collection

The study site for this project was Lake Opeongo (45°42’ N, 78°22’ W), a 5860-ha lake in southern Ontario. Smallmouth bass were presumably introduced into the lake in the 1920s and have established a self-sustaining population (Martin and Fry 1973). The collection of samples was confined to 6 km of shoreline in the Jones Bay area of the lake (Fig. 1).

Nest sites were located by snorkeling the shoreline of Jones Bay every 3 d during the 1992 spawning season (late May through mid-July). Once a nest containing eggs was located, it was marked with a numbered brick for future identification and was noted on a topographical lake map. Egg deposition was detected in a total of 202 nests (Fig. 1). From the nests that had offspring surviving to the swim-up stage (N = 116), 10 fry were randomly collected with an aquarium net and placed separately in labeled vials. All samples collected in the field were kept on dry ice and stored later at −70°C until the DNA could be extracted.

During the daylight hours of a 1-wk period in September 1992, two electrofishing surveys were conducted along the entire shoreline of the study area to collect fall YOY. Fall YOY inhabit the rocky littoral zone in Lake Opeongo. The littoral zone typically does not extend beyond 5 m from shore, and attains a maximum depth of ø 1 m. The confinement of fall YOY to this limited area near shore provided for efficient and unbiased capture using electrofishing equipment. Thus, we assumed that electrofishing allowed us to obtain a random sample of fall YOY. The second electrofishing survey provided a Peterson mark–recapture estimate of population size (Ricker 1975) and an estimate of cap-
ture efficiency. In each survey, electrofishing extended to \( \approx 1 \) km of shoreline beyond each boundary of the study area to insure capture of fall YOY that may have migrated outside the study area. Prior to returning each collected fish back into the lake, capture location was noted on a topographical lake map, and fin tissue was obtained. Fin tissues were placed separately in labeled vials and stored in a manner similar to that described above until the DNA could be extracted.

**DNA fingerprinting**

DNA of whole fry and fin tissue samples was extracted using standard phenol–chloroform procedures (Sambrook et al. 1989). Fingerprints for all samples were generated by digesting the DNA with restriction enzyme *Hae* III and electrophoretically separating the fragments through a 1% agarose gel at 60 V for 44 h. DNA was subsequently transferred to a nylon membrane and hybridized with an oligonucleotide probe (GACA), A detailed description of the methods used to generate fingerprints is presented in Gross et al. (1994).

Eight fry from the same nest were fingerprinted on the same gel to generate family-specific marks. Upon completion of fingerprinting for all YOY samples, each fall YOY fingerprint was visually compared to each family fingerprint to determine its family of origin, following the same procedures illustrated in Gross et al. (1994: Fig 6). A match was determined when the banding pattern of a fall YOY concurred with the overall banding pattern of a particular nest. Gross et al. (1994) provided verification of the ability to successfully match individuals back to their family of origin.

**Reproductive success**

Unless stated otherwise in the next section, a male was scored as reproductively successful (score = 1) if at least one of his offspring was captured at the fall YOY stage, and as unsuccessful (score = 0) if no fall YOY were captured. Variables measured to evaluate differences between successful and unsuccessful males included the guardian male fork length, guardian male age, spawning date, number of eggs within a nest, number of fry within a nest, nest depth, and the dominant substrate type both within and surrounding the nest.

Male fork length was obtained by angling each guardian male from his nest and measuring his fork length to the nearest millimeter. Male age was determined by counting the number of complete annular rings formed on scales that were obtained from each male. A given male’s spawning date was estimated as the date on which eggs were first observed in a nest, based on regular snorkeling surveys. The number of eggs in a nest was estimated using the methods of Raffetto et al. (1990). The number of fry at swim-up was estimated by visually determining the area occupied by a group of 100 swim-up fry, and then visually determining how many groups of 100 fry were in the nest.

Nest depth was measured in centimeters from the top of the substrate in the center of the nest to the water surface. Dominant substrate type within the nest and near the nest was visually categorized as belonging to one of six types: small rock (\( \leq 8 \) cm in diameter), large rock (>8 cm in diameter), macrophytes, sand, silt, and woody debris.

**Statistical analysis**

Logistic regressions (Kleinbaum 1994) were performed to determine whether any of the variables measured (except the number of eggs and fry in the nest) were significant predictors \((P < 0.05)\) of reproductive success. We tested a full model with all possible two-way interactions, and also tested all possible reduced models. Because data on the number of eggs and fry were only available for a portion of the nests, an independent \( t \) test, instead of logistic regression, was used to determine differences between successful and unsuccessful males for these traits. In addition to analyzing the data in terms of whether or not males were successful, two statistical procedures were used to analyze the reproductive success of males based on their relative contribution up to the fall YOY stage. First, the influence of each of the variables on the success of males was analyzed by performing a stepwise linear regression, with the response being the number of fall YOY produced per male. Second, a goodness-of-fit test was conducted to determine if the number of fall YOY produced per male fit the expected Poisson distribution.

In a population, random variation of family size should give rise to a Poisson distribution (Falconer 1989).

**RESULTS**

**Fall YOY and nest of origin**

A total of 283 fall YOY were captured during the two electrofishing surveys. The population size in the study area was estimated to be \( \approx 950 \) fall YOY, based on Peterson mark–recapture analysis. Thus, the capture efficiency of fall YOY was approximately 30% of the total estimated population, indicating that we obtained a representative sample of the population in the study area.

Of the 283 fall YOY captured, 226 were successfully fingerprinted and 57 fish samples (i.e., a random subsample) were lost due to technical complications. The origins of the 226 fall YOY (Table 1) were determined.

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total captured</td>
<td>283</td>
</tr>
<tr>
<td>No fingerprint generated</td>
<td>57</td>
</tr>
<tr>
<td>Family of origin identified</td>
<td>139</td>
</tr>
<tr>
<td>Family of origin narrowed to two possible nests</td>
<td>23</td>
</tr>
<tr>
<td>Originated from outside study area</td>
<td>64</td>
</tr>
</tbody>
</table>

Table 1. Summary of the origins of fall YOY based on the comparison of their fingerprints to family fingerprints.
by comparing each individual fingerprint to the family fingerprints. A family of origin (i.e., a male parent) could be assigned to 139 of the fall YOY. These fish were subsequently used in the analysis of reproductive success.

A family of origin for 23 fall YOY was narrowed down to two possible families, but could not be further resolved due to the poor quality of these YOY fingerprints (the banding pattern was distorted or faint), which increased the difficulty of comparing them to family fingerprints. Of the 46 possible families from which these fall YOY could have originated (two possible families for each YOY), all except one male was already identified as successful based on the 139 assignments of family origin. Reproductive success, therefore, was analyzed both with and without data of the one potentially additional successful male.

Sixty-four fall YOY appeared to have originated from outside of the study area, because they could not be matched to any of the family fingerprints. A majority of these fish (57) were captured along the boundaries of the study area (both within and beyond the study area), which would explain the lack of a match. However, it is possible that some of these individuals were survivors from a family within the study area for which we had presumed the deaths of all the offspring at a very early life stage, and thus had not generated a family fingerprint.

Reproductive success

Fig. 1 depicts the location of nest sites for both successful and unsuccessful males in Jones Bay. A total of 202 males had nests containing eggs during the 1992 spawning season. Of these males, 116 (57.4%) had offspring who survived to the swim-up fry stage. Based on the assigned nest of origin for 139 fall YOY, 56 males had at least one of their offspring survive to the fall YOY stage (Fig. 1; Table 1). These successful males represented 27.7 and 48.3% of the total number of males who had offspring at the embryo and fry stage, respectively.

The average number of sampled fall YOY produced per successful male was 2.5 ± 3.0 fish, with one male producing 19 fish (Fig. 2). The goodness-of-fit test indicated that the distribution of the observed number of males in Jones Bay who produced a particular number of fall YOY did not fit the theoretical Poisson distribution ($P < 0.001$). Thus, it appears that the number of offspring who survived to the fall YOY stage per male was not purely random, but rather influenced by one or more biological or environmental variables. This hypothesis is further supported by the observation that 11 males (5.4% of all spawning males and 19.7% of the successful males) produced 54.7% of the captured fall YOY. However, a stepwise linear regression analysis suggested that there was no relationship ($P > 0.05$) between the number of fall YOY produced by a male and the variables assessed in this study. Results from the logistic regression analysis indicated that none of these variables was a significant predictor ($P > 0.05$) of a male’s reproductive success up to the fall YOY stage of his offspring. In addition, the independent $t$ test did not detect significant differences between successful and unsuccessful males in the number of eggs ($t = 0.177, df = 79, P = 0.860$) or fry ($t = 1.039, df = 55, P = 0.303$) contained in their nests. An additional analysis of the data that included the one potentially successful male (from the group matched to two families) found that inclusion of this male did not affect the results (results not shown).

Discussion

Generation of family-specific DNA fingerprints made it feasible to monitor the survival of offspring from male smallmouth bass in Lake Opeongo, and thereby allowed us to estimate and evaluate reproductive success under natural conditions. If our finding, that only a small percentage of males produced the fall YOY, is a common phenomenon across years, this could have significant implications for the population dynamics of the smallmouth bass in Lake Opeongo. Our finding, that 5.4% of all spawning males in Jones Bay produced more than half of the captured fall YOY, suggests that even a smaller fraction of the spawning fish may actually produce a given year-class of adults. If recruitment depends on the success of only a few males each year, then the productivity of smallmouth bass populations is extremely vulnerable to both natural and anthropogenic impacts. Presently, natural resource managers cannot differentiate, a priori, the few highly successful males from the larger total number of nest-
ing males. Differentiating regulations that would some-
how implement a higher standard of protection for
these few males and a lower standard for all other nest-
ing males are therefore unfeasible. Instead, our results
underscore the need to protect all spawning and brood-
guarding males from anthropogenic sources of mor-
tality and nest abandonment to the fullest extent pos-
sible. This recommendation applies to the regulation
of numerous human activities that might affect the nest-
building, spawning, and brood-guarding phases. Ex-
amples include the regulation of sportfishing and other
water recreation, shoreline development, dredging, wa-
ter control structures, chemical applications, and water
discharges. Future elucidation of the factors that in-
fluence reproductive success of individual males should
lead to better predictions of population dynamics, par-
ticularly recruitment, and to improved management of
the species.

The fact that variation in brood size was nonrandom
indicates that certain biological or physical factor(s)
are responsible for the differential reproductive success
of male smallmouth bass to the fall YOY life stage in
Lake Opeongo. However, none of the variables mea-
sured in this study were significant predictors of male
reproductive success based on survival to fall YOY. A
significant relationship might not have been detected
due to a small number of males observed with more
than four fall YOY (6 of 56 males; Fig. 2). In addition,
none of our measured variables could significantly pre-
dict the success of individual males up to the fry swim-
up stage (results not shown). The latter result differed
from prior studies on smallmouth bass that found sev-
eral variables (including variables measured in the
present study) to be important predictors of male re-
productive success at the swim-up fry stage. In Lake
Erie, for example, Goff (1986) found that strong winds
during egg incubation and coarse nest substrates were
negatively correlated with male reproductive success.
Wiegmann et al. (1992) found that nest substrate and
the number of eggs spawned in the nest significantly
influenced male reproductive success in Nebish Lake,
Wisconsin. Reynolds (1990) reported that male length
and distance to upstream cover (in 1988) and spawning
date and substrate velocity (in 1989) significantly influ-
enced reproductive success of smallmouth bass in
two Tennessee streams. In a Virginia stream, a variety
of variables were significant predictors of male success,
including higher flow at nest construction, higher mean
water temperatures, lower mean discharge during in-
cubation, lower distance to shore, and higher rates of
male aggression (Lukas and Orth 1995). Ridgway and
Shuter (1994) found that, during the 1989 spawning
season in Lake Opeongo, males receiving supplemental
food had increased reproductive success relative to un-
fed males. They hypothesized that supplemental feed-
ing relieved energy constraints that may have resulted
from the competition for food among a large year-class
of fish. These widely varying results may be due to
interannual and site variation in the suite of factors
influencing reproductive success to the swim-up stage,
and make it less surprising that our results differed from
previous studies.

Factors affecting reproductive success of males up
to the fry swim-up stage may not be relevant for the
success of males based on later life stages of their
progeny. Prior studies have shown that a large pro-
portion of a population’s males can be successful at
rearing offspring to the swim-up stage. For example,
success of smallmouth bass males to the point when
their offspring are swim-up fry has been reported to
range from 33–92% (Pfleiger 1966, Turner and
MacCrimmon 1970, Neves 1975, Goff 1986, Ridgway
and Friesen 1992, Lukas and Orth 1995), and was
57.4% for our study. However, only 27.7% of the males
(56) in our study successfully produced offspring that
survived to the fall YOY stage. The reduction in
the percentage of successful males coupled with the fact
that variation in family size did not occur at random
suggest that factors encountered by progeny after
swim-up are important in determining reproductive
success of males.

Two attributes of the variables we measured might
explain why they were not significant predictors of re-
productive success to the fall YOY stage. First, all of
the measured variables were closely associated with
spawning, whereas reproductive success to relatively
late life stages of progeny could be highly dependent
on variables not directly related to spawning. Second,
we discovered that YOY smallmouth bass dispersed
relatively short distances (an average of 87.63 ± 60.67
m) from their natal nest (Gross 1995). This would sug-
gest that local ecological factors (e.g., zooplankton
abundance, predator density, disease occurrence, avail-
ability of shelter, etc.) may be important in determining
the survival of offspring from a particular nest.

Numerous factors, at different critical life stages,
may ultimately control individual reproductive success.
Expansion of this research to include analysis of ad-
ditional variables and life stages after YOY should lead
to a better comprehension of which factors have the
most influence on the reproductive success of male
smallmouth bass through the ontogeny of their off-
spring. Understanding the underlying mechanisms
that influence reproductive success should lead to more
realistic population dynamics models (e.g., Jager et al.
1993). With this knowledge, managers of natural pop-
ulations of smallmouth bass will be better able to reg-
ulate fishing rates and manage the habitat to ensure that
all individuals spawning in a given year have the op-
portunity to reproduce successfully. Finally, we have
demonstrated that molecular genetic techniques, such
as DNA fingerprinting, provide powerful tools to ad-
dress hitherto intractable questions about the ecology
of fishes.

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**Literature Cited**


