

Morphometric changes associated with the reproductive cycle and behaviour of the intertidal-nesting, male plainfin midshipman *Porichthys notatus*

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Morphometric changes in body condition, liver, sonic muscle and gonadal development associated with the annual reproductive cycle and behaviour of the intertidal-nesting male plainfin midshipman *Porichthys notatus* were investigated. Body condition of type I males rapidly increased during the pre-nesting (PN) period, peaked at the beginning of the summer nesting cycle and then gradually declined to lowest levels during the non-reproductive (NR) period. The gonado-somatic index of type I males peaked during PN and then declined during the summer nesting cycle to lowest levels at the end of the nest cycle and during NR. Indices of sonic muscle and liver of type I males were lowest during NR, gradually increased during PN and then peaked during the summer nesting cycle. Results indicate that body condition and fecundity of type I males were positively correlated with body mass at the end of the nest cycle. These findings as they relate to the annual reproductive cycle and behaviour of the type I male *P. notatus* are discussed.

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Key words: batrachoididae; condition factor; gonadal development; liver; sonic muscle.

INTRODUCTION

Marine teleosts of the family Batrachoididae that include the toadfishes and midshipman fishes have become important models for investigating neural and endocrine mechanisms of acoustic communication shared by all vertebrates (Fay & Simmons, 1999; Bass & McKibben, 2003; Bass, 2006; Forlano *et al.*, 2007). Although the plainfin midshipman *Porichthys notatus* Girard has been the subject of recent investigations of auditory neurophysiological plasticity related to the annual reproductive cycle (Sisneros & Bass, 2003; Sisneros *et al.*, 2004a), very little information is available on the morphological correlates associated with seasonal periodicity of the reproductive cycle and behaviour of this batrachoidid species. *Porichthys notatus* is primarily a deep-water fish found along the eastern

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Pacific coast from Sitka, Alaska, U.S.A., to Magdalena Bay, Mexico (Hubbs, 1920; Arora, 1948; Miller & Lea, 1972; Walker & Rosenblatt, 1988). This nocturnally active fish spends the day buried in sandy substrata at depths up to 300 m (Miller & Lea, 1972) and then at night rises in the water column to feed on small crustacean larvae, other zooplankton and small fishes (Eigenmann, 1892; Hubbs, 1920; Arora, 1948). *Porichthys notatus*, like other batrachoidids in the genus *Porichthys*, is bioluminescent with more than several hundred small dermal photophores arranged in several distinct and species-specific rows over the head and body (Greene, 1899). The mechanism and potential function of bioluminescence in *Porichthys* have received much attention over the past few decades (Crane, 1965, 1981; Tsuji *et al.*, 1971; Thompson *et al.*, 1988*a, b*; Mensinger & Case, 1991; Harper & Case, 1999).

During late spring and summer, *P. notatus* migrate from deep offshore into the subtidal and intertidal zones to court and spawn. *Porichthys notatus* have three adult reproductive morphs that include females and two male morphs: types I and II, each type has divergent behavioural and reproductive tactics (Bass, 1996). Breeding type I males build and defend nests that are positioned under rocky shelters from which they produce relatively long duration (>1 min) advertisement calls at night to attract females for spawning (Bass *et al.*, 1999). Reproductive females use their auditory system to detect and locate 'singing' type I males that produce the seasonal advertisement call. In contrast to type I or 'nesting' males, type II males use an alternative reproductive tactic that does not require them to build nests nor acoustically court females. Instead, type II males satellite and 'sneak' spawn to steal fertilizations from type I males that are actively courting females (Brantley & Bass, 1994). After depositing all their eggs into a single nest, females that have spawned leave the seasonal intertidal breeding grounds and return to deep offshore sites. Type I males remain in their intertidal nests and continue to court and spawn with other females over the course of the breeding season until their nests are filled with multiple clutches of eggs and embryos (DeMartini, 1988; Brantley & Bass, 1994; Bass, 1996). In this species, type I males provide all the parental care which often consists of brushing and fanning the eggs to keep them clean (Arora, 1948). Furthermore, parental type I males will vigorously guard and defend their nests from egg predators until their young become free swimming *c.* 30–40 days post-fertilization (Brantley & Bass, 1994).

The primary goal of this study was to investigate morphometric changes associated with the annual reproductive cycle and behaviour of the type I male *P. notatus*. A particular interest was to determine how morphometric indices of body condition, liver, sonic muscle and gonadal development change from the non-reproductive to the reproductive breeding season. Additional interests include how such morphometric indices change over the course of the summer nesting season, when type I males are known to seasonally produce their metabolically expensive advertisement call, are exposed daily at low tide to fluctuating stressful conditions of temperature and reduced O₂ availability, and presumably undergo starvation since they are not known to feed during the breeding period (Arora, 1948). Thus, an examination was made to determine the morphometric changes of intertidal-nesting type I males across three phases of the summer nesting cycle. The findings are interpreted as they relate to the annual reproductive cycle and behaviour of type I males.

MATERIALS AND METHODS

ANIMALS AND SEASONAL COLLECTION PERIODS

A total of 67 adult type I male *P. notatus* [mean \pm s.d. standard length (L_S) = 212 \pm 49 mm] were collected during three time periods that corresponded to seasonal fluctuations in *P. notatus* reproductive biology and behaviour from May 2005 to January 2007 in western Washington, U.S.A. The three seasonal time periods were based on an earlier description by Sisneros *et al.* (2004b) and were defined as: (1) non-reproductive (NR), which occurred in the autumn and winter months when type I males had the lowest gonado-somatic index (I_G ; the ratio of gonad mass to body mass) with no mature sperm present in testes and were collected by otter trawl at the deepest depths offshore during November 2006 (mean \pm s.d. collection depth = 100 \pm 20 m) and January 2007 (mean \pm s.d. collection depth = 74 \pm 7 m) in Puget Sound near Edmonds, Washington, U.S.A. (R.V. *Kittiwake*, Bio-Marine Enterprises); (2) pre-nesting (PN), which occurred in late winter and early spring when type I males undergo seasonal gonadal recrudescence, have the greatest I_G and were collected by otter trawl at the shallowest depths offshore during February 2006 (mean \pm s.d. collection depth = 56 \pm 20 m) in Puget Sound near Blake Island, Washington, U.S.A. (F.V. *Chasina*) and (3) the nesting period, which occurred in the late spring and summer (late April to early August; DeMartini, 1988, 1991) when type I males were collected by hand from intertidal nests during low tide at depths <0.25 m in the same general geographical location used in other previous studies near Seal Rock in Brinnon, Washington, U.S.A. (DeMartini, 1988, 1991; Lee & Bass, 2004, 2006). The nesting period was further divided into three stages (based on the nest cycle): nesting 1 (N1), which was defined as an early stage of the nest cycle when males had nests with only fresh eggs that were collected during May 2005 and 2006; nesting 2 (N2), which was an intermediate stage of the nest cycle when males had nests with broods of at least 50% non-pigmented, recently hatched embryos that were collected during June 2005 and July 2006 and nesting 3 (N3), which was a late stage of the nest cycle when males had nests with broods of at least 75% well-developed pigmented embryos that were collected during July and August 2005 and July 2006.

Several criteria have been used over the past 20 years to distinguish the two male morphs (type I v. type II) during the reproductive breeding season. Such criteria include body size, I_G , sonic-muscle colouration and sonic-muscle somatic index (I_{SM} ; the ratio of sonic swimbladder muscle to body mass) (Bass, 1996). Using the above-mentioned criteria, the males collected in this study during the spring and summer reproductive season were unambiguously type I males. It is recognized, however, that during NR periods a reduction in I_G (Sisneros *et al.*, 2004b) and a regression of sonic-muscle size may preclude the use of these traits for distinguishing type I males. Therefore, only body size could be used as a reliable trait for identifying type I males outside the spring and summer breeding season. All the males ($L_S \geq 117$ mm) collected outside the breeding season in this study were well above the previously reported size range of anatomically identified type I males ($L_S \geq 105$ mm) collected in previous studies (Brantley *et al.*, 1993; Grober *et al.*, 1994; Bass *et al.*, 1996; Foran & Bass, 1998). Over the past 20 years, only 2% of type II males ($n = 431$) sampled have been ≥ 117 mm L_S (Brantley *et al.*, 1993; Grober *et al.*, 1994; Bass *et al.*, 1996; Foran & Bass, 1998; A.H. Bass, pers. comm.). Thus, the probability that type II males were collected during the winter NR period in this study is thought to be very low. Type II males may yet be found in shallower waters during the NR period in sites close to the intertidal zone.

SAMPLE COLLECTION

The L_S , body mass (M), gonad mass (M_G), liver mass (M_H) and sonic-muscle mass (M_{SM}) were recorded for each fish. The stomach and intestine contents were also noted and weighed (M_{GC}) for the reproductive nesting type I males. The mass of all biological

material was measured to the nearest 0.01 g. Based on the recorded body morphometrics, several indices were calculated to assess the condition of the type I males. Body condition or condition factor was expressed as Fulton's condition factor (K) (Fulton, 1904) derived from: $K = 10^5 M_B L_S^{-3}$, where M_B is somatic body mass (g) [$M - (M_G + M_{GC})$] and L_S is in mm (Bagenal, 1978). The hepato-somatic index (I_H) (Cailliet *et al.*, 1986) was calculated from the formula: $I_H = 100 M_H M_B^{-1}$.

The gonado-somatic index (I_G) (Tomkins & Simmons, 2002) was used to describe reproductive state of the testes with the formula: $I_G = 100 M_G M_B^{-1}$. The gonads from type I males collected during the reproductive spawning period were preserved for histological examination. The sonic-muscle somatic index (I_{SM}) was used to describe the seasonal development of the sonic swimbladder muscles from the equation: $I_{SM} = 100 M_{SM} M_B^{-1}$.

The sonic muscles and swimbladders from non-reproductive and reproductive type I males were preserved in 10% seawater-buffered formalin. Later, the sonic muscles were dissected and separated from the swimbladder to obtain M_{SM} .

In order to determine if type I males were feeding during the reproductive nesting season, the stomach and the intestines of nesting type I males collected during N1–N3 were extracted and then examined under a dissecting microscope. The stomach and intestine contents were examined ($\times 400$ magnification) and the presence of small crustaceans (crabs), gastropods, *P. notatus* eggs and embryos was noted and the total wet mass of the stomach and intestine contents (M_{GC}) was recorded. A feeding intensity index (I_{FI}) was calculated from the following: $I_{FI} = 100 M_{GC} (M_B - M_{GC})^{-1}$.

A detailed analysis of the diet composition of nesting type I males was not an aim of this study. Instead, the focus of this analysis was a more limited investigation to determine the presence or absence of prey items and their frequency of occurrence.

The number of offspring produced by type I males collected during N3 of the nest cycle was determined by counting the number of viable late-staged embryos found in the nests. Late-stage embryos were identified as being well developed, pigmented and having a reduced amount of yolk. Individual embryos were removed from the nests and counted to determine the number of offspring reared and guarded by the nesting type I male during N3.

TISSUE HISTOLOGY

Testes were collected from animals and initially stored in 5% buffered formalin and then later preserved in 10% buffered formalin. Prior to sectioning, testes were rinsed in distilled water over night and then sectioned on a freezing microtome into 60 μm sections. The tissue sections were then mounted onto slides, stained with cresyl violet and dehydrated in a graded series of alcohols and xylene. Following the final xylene rinse, the slides were coverslipped with Permount, allowed to dry and then examined under light microscopy.

STATISTICAL ANALYSES

The effect of seasonal period (non-reproductive, pre-nesting and nesting) on K , I_G , I_H and I_{SM} were determined by a one-way ANOVA followed by the *post hoc* Tukey's honest significant difference (HSD) test for pair-wise multiple comparisons (Zar, 1999). The Levene and the Brown–Forsythe tests were used to test the homogeneity of variances. When data sets did not meet the ANOVA assumptions of homogeneity of variances, the data were \log_{10} transformed and then a one-way ANOVA followed by the Tukey's HSD test for pair-wise multiple comparisons was performed. When the \log_{10} transformed data still did not meet the ANOVA assumption of equal variances, a one-way ANOVA with Tukey's HSD test for multiple comparisons was performed but the α level was set to 0.01 to reduce the possibility of a type II error. Since not all of the morphometric sample data (*i.e.* I_G data) were normally distributed, both the means and the s.d. are reported in Table I to facilitate comparisons with other

published studies on this and other species. Due to the relative low samples reported in this study, a *post hoc* power of an ANOVA (Zar, 1999) for the effect of seasonal period (non-reproductive, pre-nesting and nesting) on K , I_G , I_H and I_{SM} was performed. In all cases, the power of the ANOVA was estimated to be ≥ 0.82 . Associations of K and M_B , I_{SM} and L_S of male fish, and the number of offspring and parental body mass were determined using a Pearson's correlation and linear regression using the software programme the Statistica for Windows (StatSoft, Inc., Tulsa, OK, U.S.A.). For all tests, unless otherwise noted, α was set at 0.05.

RESULTS

CHANGES IN MORPHOMETRIC INDICES OF BODY CONDITION, GONAD, LIVER AND SONIC MUSCLE RELATED TO THE TYPE I MALE REPRODUCTIVE CYCLE

Type I male *P. notatus* showed seasonal changes in K , I_G , I_H and I_{SM} that varied with the reproductive cycle and behaviour (Table I). Type I males showed seasonal variation in K that peaked during N1 of the nesting cycle (Fig. 1). A maximum K of 1.919 was recorded for a 262 mm male collected during N1 in May. The lowest K was 1.053 recorded for a 205 mm male collected during N3 in August. Although there was no difference in K between males of N1 (mean = 1.552, $n = 16$) and N2 (mean = 1.468, $n = 12$), the mean K for males collected during N1 was 1.24 times greater than males collected during both NR (mean = 1.247, $n = 18$) and N3 (mean = 1.291, $n = 16$), while the mean K for males collected during N2 was 1.18 times greater than the K of males collected during NR and no different than the K of males collected during N3 (one-way ANOVA, Tukey's HSD test, F , d.f. = 4, 62, $P < 0.001$). Furthermore, there was no significant difference between the median K of type I males collected during NR and N3. Thus, K was highest during N1 and N2 of the nesting cycle and lowest during the non-reproductive season and during N3 of the nesting cycle.

In addition to K , the stomach and intestine contents of nesting type I males were examined for the presence of food during N1–N3 to determine whether type I males feed while guarding and providing parental care for young in their nests. The I_{FI} of nesting males was $< 0.4\%$ (mean = 0.31%) for the 44 nesting males that were examined throughout the nest cycle (N1–N3). Of the identifiable prey items found in the stomachs and intestines of nesting males, *P. notatus* eggs and embryos had the highest frequency of occurrence in the stomach and intestines of type I males throughout the nest cycle (Table II). During N1, *P. notatus* eggs were found in 75% of the nesting males examined. During N2, however, *P. notatus* eggs and embryos were found in *c.* 42 and 25% of type I nesting males, respectively. In the last stage of the nesting cycle or N3, *P. notatus* eggs and embryos were found in *c.* 12.5 and 31% of the nesting males, respectively. The only other identifiable prey items found were small shrimp and crabs (Crustacea, Decapoda: Caridea and Brachyura), which were found in $\leq 8\%$ of the stomachs and intestines of nesting males during N1–N3 (Table II). Approximately 29% (13 of the 44 animals) of the nesting males examined during N1–N3 had empty stomachs and intestines. Although there was no difference in I_{FI} among type I males during N1–N3 [one-way ANOVA,

TABLE I. Morphometric measurements standard length (L_S) and somatic mass (M_B) and of body condition (K), gonado-somatic index (I_G), hepato-somatic index (I_H) and sonic-muscle somatic index (I_{SM}) for type I male *Porichthys notatus*

| Month | L_S (mm) | | M_B (g) | | K | | I_G | | I_H | | I_{SM} | |
|---|---|-----|---|-----|---|-----|---|-----|---|-----|---|-----|
| | Mean \pm s.d. (minimum, medium, maximum) | n |
| Non-reproductive (November 2006 and January 2007) | 143 \pm 21 (11.7, 14.0, 19.4) | 18 | 39.0 \pm 19.9 (20.3, 35.5, 92.1) | 18 | 1.247 \pm 0.081 (1.074, 1.264, 1.358) | 18 | 0.62 \pm 1.19 (0.03, 0.11, 3.29) | 18 | 2.53 \pm 0.33 (2.06, 2.57, 2.94) | 11 | 0.38 \pm 0.12 (0.28, 0.34, 0.76) | 18 |
| Pre-nesting (February 2006) | 216 \pm 22 (19.4, 21.1, 25.1) | 5 | 144.8 \pm 40.8 (95.9, 142.8, 198.6) | 5 | 1.384 \pm 0.155 (1.221, 1.338, 1.609) | 5 | 2.73 \pm 0.84 (1.54, 2.92, 3.67) | 5 | 2.72 \pm 0.81 (1.92, 2.75, 4.00) | 5 | 0.87 \pm 0.23 (0.56, 0.80, 1.16) | 5 |
| Nesting stage I (May 2005 and 2006) | 243 \pm 25 (17.9, 25.1, 27.5) | 16 | 236.9 \pm 76.9 (80.4, 241.5, 387.1) | 16 | 1.552 \pm 0.173 (1.269, 1.535, 1.919) | 16 | 1.94 \pm 0.48 (0.92, 1.97, 3.01) | 16 | 2.83 \pm 0.97 (1.77, 2.63, 5.65) | 16 | 1.14 \pm 0.21 (0.80, 1.14, 1.41) | 16 |
| Nesting stage II (June 2005 and July 2006) | 236 \pm 31 (18.7, 24.1, 27.7) | 12 | 206.1 \pm 83.8 (81.7, 204.1, 353.9) | 12 | 1.468 \pm 0.124 (1.233, 1.488, 1.652) | 12 | 1.15 \pm 0.78 (0.69, 0.92, 3.35) | 12 | 3.44 \pm 0.92 (2.34, 3.11, 5.72) | 12 | 1.18 \pm 0.21 (0.93, 1.11, 1.55) | 12 |
| Nesting stage III (July and August 2005 and July 2006) | 239 \pm 22 (20.5, 24.3, 26.8) | 16 | 184.0 \pm 59.1 (91.9, 180.9, 308.3) | 16 | 1.291 \pm 0.222 (1.053, 1.240, 1.948) | 16 | 1.05 \pm 0.60 (0.40, 0.96, 2.69) | 16 | 3.69 \pm 0.90 (2.50, 3.45, 5.52) | 16 | 1.09 \pm 0.13 (0.81, 1.11, 1.30) | 16 |

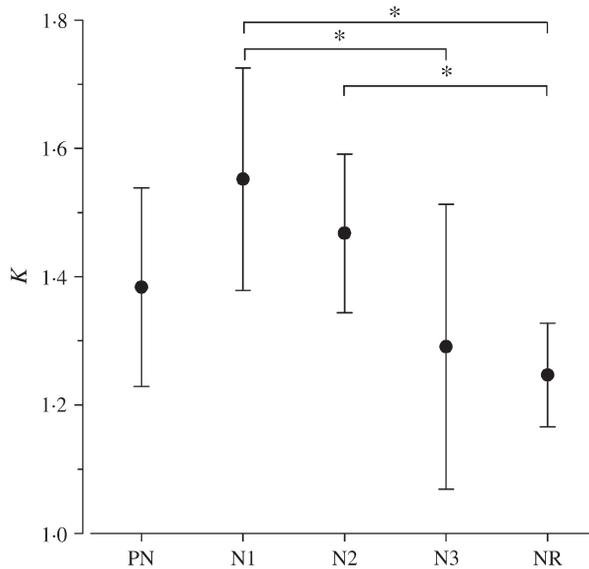


FIG. 1. Seasonal variation in body condition (K , Fulton's condition factor) of wild-caught type I male *Porichthys notatus* collected from Puget Sound and the Hood Canal, WA, U.S.A., during the non-reproductive (NR) and pre-nesting (PN) periods and three stages (N1–N3) of the nest cycle during the reproductive period between May 2005 and January 2007. Values are means \pm s.d. Lines with * link periods that are significantly different (one-way ANOVA, Tukey's HSD test, $P < 0.001$).

$P > 0.01$ for $\log_{10}(x + 1)$ transformed data with unequal variances], the relatively low I_{FI} (< 0.7) for N1–N3 type I males indicates that nesting males feed very little during the nest cycle.

TABLE II. The frequency of occurrence (% F) of the dietary items found in the stomachs and intestines and the average feeding intensity index (I_{FI}) of type I male *Porichthys notatus* during N1–N3 of the summer nest cycle

| Nesting phase (N1–N3) and dietary items | % F | I_{FI} (mean \pm s.d.) |
|--|-------|----------------------------|
| N1 ($n = 16$) | | 0.64 \pm 0.83 |
| <i>Porichthys notatus</i> eggs | 75.0 | |
| Crustaceans (Crustacea, Decapoda: Caridea and Brachyura) | 6.2 | |
| Unidentified material | 6.2 | |
| N2 ($n = 12$) | | 0.13 \pm 0.09 |
| <i>Porichthys notatus</i> eggs | 41.7 | |
| <i>Porichthys notatus</i> embryos | 25.0 | |
| Crustaceans (Crustacea, Decapoda: Caridea and Brachyura) | 8.3 | |
| Unidentified material | 41.7 | |
| N3 ($n = 16$) | | 0.14 \pm 0.21 |
| <i>Porichthys notatus</i> eggs | 12.5 | |
| <i>Porichthys notatus</i> embryos | 31.2 | |
| Crustaceans (Crustacea, Decapoda: Caridea and Brachyura) | 6.2 | |
| Unidentified material | 43.7 | |

The I_H of type I males steadily increased from PN to N3 of the nest cycle, but then decreased during NR (Fig. 2). The I_H was highest during N2 and N3 of the nesting cycle with a maximum I_H of 5.72 recorded for a 207 mm male collected during N2 in July (Table I). Although there was no difference in I_H among males collected during N2 (mean \pm s.d. = 3.44 ± 0.92 , $n = 12$) and N3 (mean \pm s.d. = 3.69 ± 0.90 , $n = 16$), the mean I_H of males collected during N3 was *c.* 1.3–1.5 times greater than the I_H of males collected during NR (mean \pm s.d. = 2.53 ± 0.33 , $n = 11$) and N1 (mean \pm s.d. = 2.83 ± 0.97 , $n = 16$), while the I_H of males collected during N2 was not significantly different from PN (mean \pm s.d. = 2.72 ± 0.81 , $n = 5$), NR or N1 (one-way ANOVA, Tukey's HSD test, F , d.f. = 4, 55, $P < 0.01$).

The I_G of type I males varied significantly across different periods of the reproductive cycle and was greatest during PN and N1 (Fig. 3). The maximum I_G of 3.67 was recorded for a 211 mm male collected during PN in February and the lowest GSI was 0.03 recorded for a 141 mm male collected during NR in January (Table I). Although there was no difference in I_G among males collected during PN (mean = 2.73, $n = 5$), N1 (mean = 1.94, $n = 16$) and N2 (mean = 1.15, $n = 12$) and N3 (mean = 1.05, $n = 16$), the mean I_G for males collected during PN and N1 was 4.4 and 3.1 times greater than the I_G of males collected during NR (mean = 0.62, $n = 18$), respectively (one-way ANOVA, Tukey's HSD test, F , d.f. = 4, 62, $P < 0.001$). Despite no significant difference in I_G among the time periods of PN, N1 and N2, histological examination of

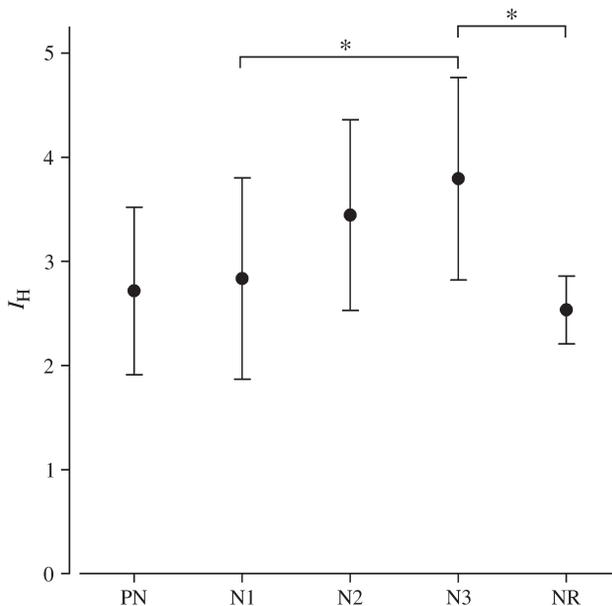


FIG. 2. Seasonal variation in the hepato-somatic index (I_H) of type I male *Porichthys notatus* collected from Puget Sound and the Hood Canal, WA, U.S.A., during the non-reproductive (NR) and pre-nesting (PN) periods and three stages (N1–N3) of the nest cycle between May 2005 and January 2007. Values are means \pm s.d. Lines with * link periods that are significantly different (one-way ANOVA, Tukey's HSD test, $P < 0.01$).

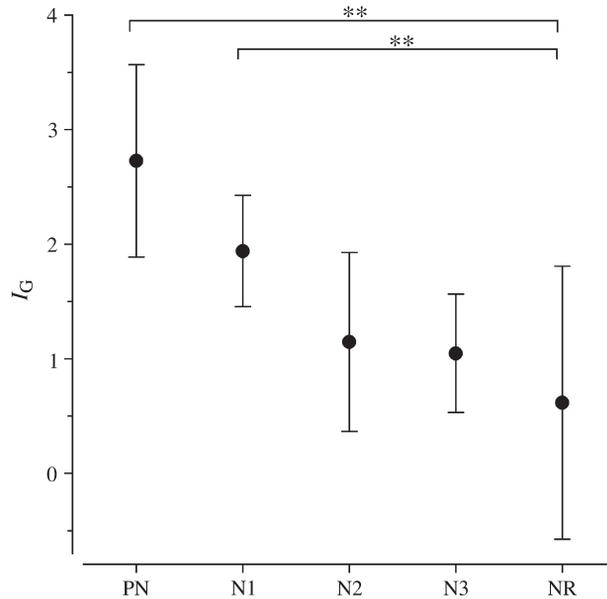


FIG. 3. Seasonal variation in gonado-somatic index (I_G) of type I male *Porichthys notatus* collected from Puget Sound and Hood Canal, WA, U.S.A., during the non-reproductive (NR) and pre-nesting (PN) periods and three stages (N1–N3) of the nest cycle between May 2005 and January 2007. Values are means \pm s.d. Lines with **link periods that are significantly different (one-way ANOVA, Tukey's HSD test, $P < 0.001$).

20 representative testes from PN ($n = 5$), N1 ($n = 3$), N2 ($n = 2$) and N3 ($n = 5$) revealed that the testes of type I males from PN contained multiple types of germ cells that included spermatocytes, spermatids and spermatozoa (mature sperm), whereas the testes from males collected during N1 had a homogeneous population of mature sperm (Fig. 4). The testes of males collected during N2 and N3 revealed high incidence of mature sperm but also spermatocytes and spermatids. In contrast, the regressed testis from males collected during NR had primarily spermatogonia with low incidence of spermatocytes (Fig. 4).

Type I males also showed seasonal changes in I_{SM} that significantly varied with season period and reproductive behaviour (Fig. 5). The I_{SM} was highest during the nesting cycle (N1–N3) and lowest during the non-reproductive season. The maximum I_{SM} of 1.55 was recorded for a 247 mm male collected during N2 in June and the lowest I_{SM} was 0.28 recorded for a 123 mm male collected during January (Table I). Although there was no difference in I_{SM} of males collected during N1 (mean \pm s.d. = 1.14 ± 0.21 , $n = 16$), N2 (mean \pm s.d. = 1.18 ± 0.21 , $n = 12$) and N3 (mean \pm s.d. = 1.09 ± 0.13 , $n = 16$), the I_{SM} of nesting males (N1–N3) was *c.* 2.9–3.1 times greater than the I_{SM} of males collected during NR (mean \pm s.d. 0.38 ± 0.12 , $n = 18$), while the I_{SM} of N1 and N2 males was *c.* 1.3–1.4 times greater than that of PN males (mean \pm s.d. 0.87 ± 0.23 , $n = 5$) and there was no difference in the I_{SM} between N3 and PN type I males (one-way ANOVA, Tukey's HSD test, F , d.f. = 4, 62, $P > 0.05$). Furthermore, the I_{SM} of males collected during PN was *c.* 2.3 times greater than the I_{SM} of

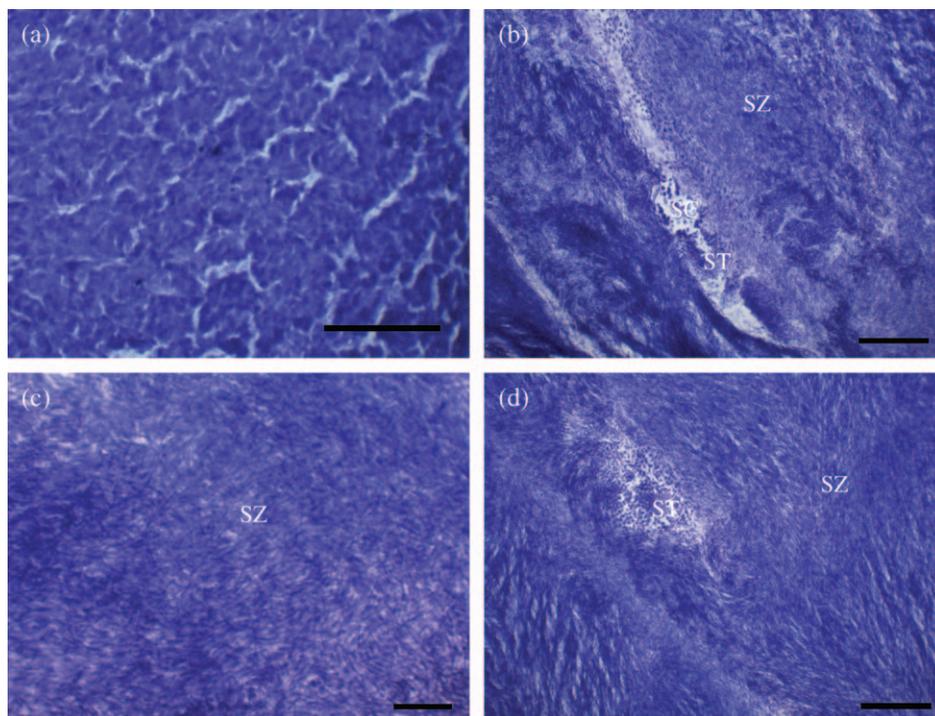


FIG. 4. Representative stages of testis development during the annual reproductive cycle of type I male *Porichthys notatus*. (a) Regressed testis from the non-reproductive winter period (early January); note only spermatogonia are present. Scale bar: 100 μm . (b) Testis from the pre-nesting period (late February); multiple types of germ cells are interspersed among mature sperm. Scale bar: 100 μm . (c) Testis from the first stage (N1) of the nest cycle (late May) contains a homogeneous population of mature sperm. Scale bar: 200 μm . (d) Testis that is representative of both the second and the third stage (N2 and N3) of the nest cycle (June to July) that contains multiple types of germ cells that include spermatocytes, spermatids and mature sperm. Scale bar: 100 μm . SC, spermatocytes; ST, spermatids; SZ, spermatozoa.

males collected during NR. Sonic-muscle size increased most during the pre-nesting period and was coincident with the greatest increase in I_G .

The I_{SM} of nesting males (N1–N3) was negatively correlated with male L_S (Fig. 6) ($r = -0.56$; $H_0: \beta = 0$; $t = -4.42$; $P < 0.001$). A linear relationship was identified for summer nesting type I males collected during N1–N3 (Fig. 6).

RELATIONSHIPS OF BODY CONDITION AND NUMBER OF OFFSPRING PRODUCED WITH BODY MASS OF TYPE I MALES DURING N3

Among type I males collected during N3, K was positively correlated with M_B (Fig. 7) ($r = 0.50$; $H_0: \beta = 0$; $t = 2.18$; $P < 0.05$). A significant linear relationship between K and M_B was identified for type I males collected during N3 of the nest cycle. In addition, the relationship between the number of

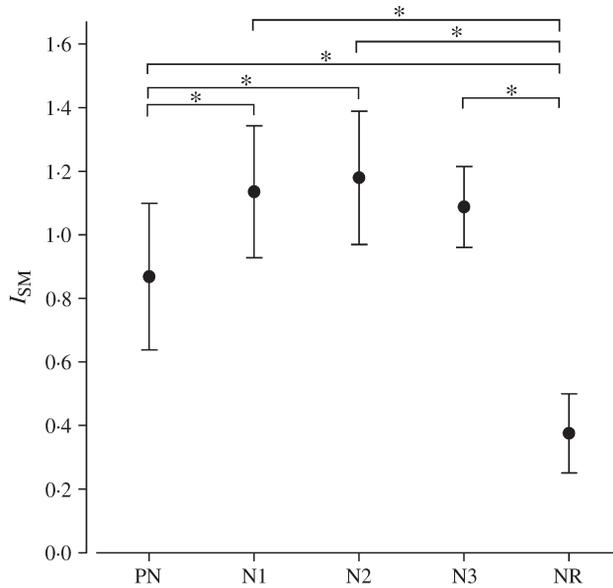


FIG. 5. Seasonal variation in the sonic-muscle somatic index (I_{SM}) of type I male *Porichthys notatus* collected from Puget Sound and the Hood Canal, WA, U.S.A., during the non-reproductive (NR) and pre-nesting (PN) periods and three stages (N1–N3) of the nest cycle between May 2005 and January 2007. Values are means \pm s.d. Lines with * link periods that are significantly different (one-way ANOVA, Tukey's HSD test, $P < 0.05$).

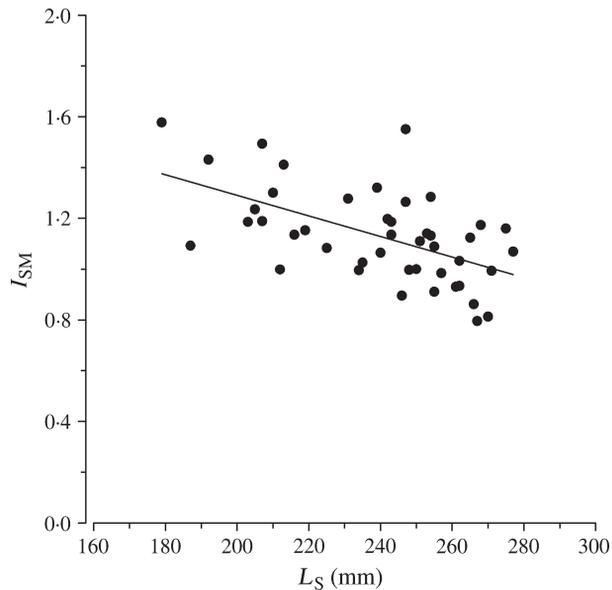


FIG. 6. The relationship between sonic-muscle somatic index (I_{SM}) and standard length (L_S) for type I male *Porichthys notatus* collected during the three stages of the summer nesting cycle. The curve was fitted by: $y = -0.004x + 2.10$.

embryos and the body mass was determined for six adult type I males (range = 207–279 mm, mean \pm s.d. = 226 ± 24 mm L_S) that were representative of male sizes found during N3 in late July near the end of the breeding summer. The number of viable late-stage embryos in the nest was highly correlated with the M_B of the nesting type I males collected during N3 of the nest cycle (Fig. 8) ($r = 0.97$; $H_0: \beta = 0$; $t = 7.64$; $P < 0.005$). A linear relationship was identified for type I males of N3. Thus, the largest type I males collected during N3 of the nest cycle had the highest K and greatest number of embryos in their nests.

DISCUSSION

The goal of this study was to investigate the morphological changes associated with the seasonal periodicity of the reproductive cycle and behaviour of type I male *P. notatus*. Of particular interest was to determine if morphometric indices such as body condition, sonic muscle and gonadal development change over the course of the summer nesting season when type I males are known to produce their energetically expensive advertisement call in the intertidal-breeding grounds where nesting males are exposed to conditions of hypoxia and starvation. This study represents a first attempt to determine the energetic consequences and the resultant change in morphometric indices of a deep-water batrachoidid that breeds and maintains their young in the shallow intertidal zone. In this discussion, the results are interpreted as they relate to the natural reproductive ecology and behaviour of this intertidal-nesting fish species.

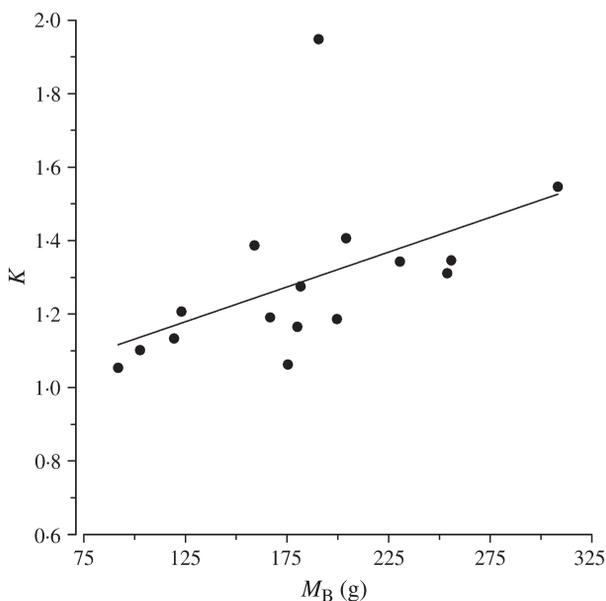


FIG. 7. The relationship between body condition (K , Fulton's condition factor) and body mass (M_B) for type I male *Porichthys notatus* collected during the last stage (N3) of the summer nesting cycle. The curve was fitted by: $y = 0.0019x + 0.94$.

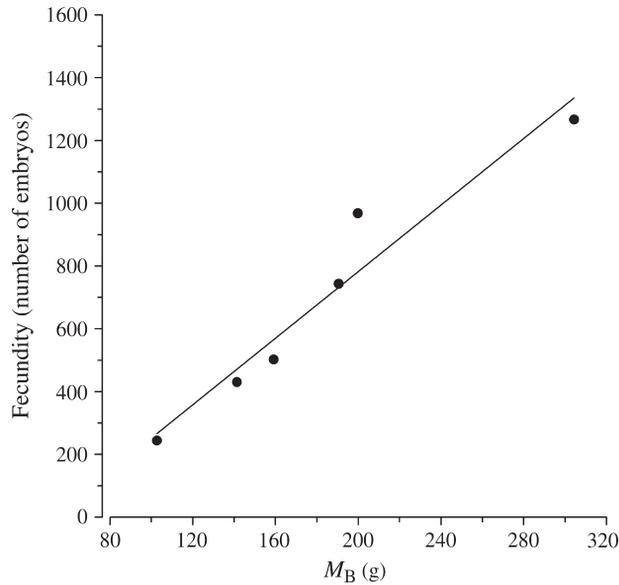


FIG. 8. The relationship between fecundity (measured as the number of embryos in the nest) and body mass (M_B) for type I male *Porichthys notatus* collected during the last stage (N3) of the summer nesting cycle. The curve was fitted by: $y = 5.30x - 277.37$.

In this study, a seasonal decrease in body condition among nesting type I males during the summer breeding season was documented. Body condition as measured by Fulton's condition factor (K) was highest at the beginning of the nest cycle (N1) when type I males only had broods of fresh eggs in their nests. The condition of type I males decreased during the nesting cycle (from N1 to N3) and was lowest at the end of the nesting cycle (N3) when males had broods of at least 75% well-developed pigmented embryos in their nests. Similar changes in K are reported for the intertidal-nesting male Lusitanian toadfish *Halobatrachus didactylus* (Bloch & Schneider), where maximum K levels are obtained prior to the breeding season followed by a decrease in K during the breeding season and then become minimal by the end of the breeding season (Modesto & Canario, 2003). The decrease in K for type I male *P. notatus* over the course of the nesting cycle is most likely due to the metabolic costs of sound production for intraspecific social communication coupled with the starvation stress of nesting males during the summer breeding season. Nesting type I males are capable of generating an advertisement call known as a 'hum' that can be very long in duration (an order of seconds to minutes and can be sustained as long as 1 h in duration; A. H. Bass, pers. comm.) and have fundamental frequencies that range from 90–100 Hz with several prominent harmonics that range up to 400 Hz. These advertisement calls are produced by nesting males during the breeding season to attract reproductively active females to their nests for spawning. The long duration hums are thought to be metabolically costly due to the apparent increase in respiration during the

production of the advertisement call under semi-natural laboratory conditions (Amorim *et al.*, 2002; Amorim, 2006; Ladich & Fine, 2006; J. Sisneros, pers. obs.). Studies of the metabolic costs of sound production in fishes are at best limited and difficult since fishes will often not produce vocalizations while in a respirometer (Amorim *et al.*, 2002). Future studies that examine oxygen consumption and the metabolic costs of sound production by type I males, especially the energetic costs of producing the advertisement call, will be extremely useful in determining the physiological energy costs of mate call production for nesting males of this species.

The stomach content data from this study also support earlier observations by Arora (1948) that nesting males are presumably starved during the breeding season when type I males guard their nests until their young become free swimming. The stomach content data indicate that food consumption by type I males was minimal during the nesting cycle. The I_{FI} of nesting males was $<0.4\%$ for all the 44 nesting males that were examined throughout the nest cycle (N1–N3). When food was present, the stomach and intestines of nesting type I males often contained either one to two small eggs or embryos. These results beg the question of whether parental type I males practice filial cannibalism regarding their own offspring or if they cannibalize the young from nearby nests of other type I males (non-kin cannibalism). Although cannibalistic behaviour is associated with a wide variety of taxa, habitats and life-history strategies of teleosts, it is particularly well represented in piscivorous and parental care-giving species (Smith & Reay, 1991). Cannibalism of the eggs and embryos, whether it be filial or non-kin, may be the only major source of food in or around the nest sites available to nesting type I males during the breeding season. Arora (1948) examined the stomach contents of type I males and found them to be mostly empty or starved and concluded that the nesting type I males must fulfil their food requirements with whatever little prey is found in or around the nest sites. The nest sites of type I males are often in close proximity to one another since they often cluster their nests together in the intertidal zone (Bass, 1996; Bass *et al.*, 1999) which could facilitate non-kin cannibalism *via* the predation of young from nearby nesting neighbours. Alternatively, nesting type I males may practice non-kin cannibalism within their own nest by discriminating the identity of their own offspring and then consuming the young from that of type II or sneaker males which may have stolen fertilizations from the nesting type I males. Recent studies of bluegill sunfish *Lepomis macrochirus* Rafinesque indicate that nest-tending parental males can assess the paternity of their own offspring using both the visual presence of sneakers during spawning and the olfactory cues released by newly hatched eggs (Neff, 2003). Once the eggs hatch the developing fry release a chemical cue (possibly in the urine) that parental males can use to distinguish their offspring from those of sneaker and satellite males (Neff & Gross, 2001; Neff & Sherman, 2002, 2003). Future studies that investigate the cannibalistic behaviour of nesting type I males using microsatellite DNA analysis to determine the genetic relatedness of the consumed offspring and prey relative to the nesting type I male would provide valuable insight in determining whether *P. notatus* practices filial or non-kin cannibalism and help determine the importance of genetic relatedness in parental care decisions for this species.

In contrast to the decrease in K of type I males during the nest cycle, the mean I_H in nesting males increased from N1 to N3 of the nest cycle. I_H is a common method used to identify seasonal changes in the liver energy reserves since mean I_H values are known to correlate with the amount of fat deposition (lipids) in the liver of fishes (Chiba & Honma, 1981; Oguri, 1985; Ando *et al.*, 1993; Brusle & Anadon, 1996). It is possible that energy reserves are stored and accumulated in the liver during the non-reproductive winter and pre-nesting spring when type I males are offshore feeding on crustacean larvae and other zooplankton (Arora, 1948). The results of an apparent increase in I_H by nesting males, however, is most likely due to a more rapid loss of body mass relative to liver mass in type I males during the nest cycle when body condition decreases and food consumption is low. An examination of the liver size from type I males that are similar in L_S , during different stages of the nest cycle will be necessary to determine the significance of the nesting male I_H increase during the breeding summer.

Results from this study demonstrate clear seasonal patterns of gonadal development associated with the reproductive season and behaviour of type I males. The annual I_G cycle in type I males begins with a maximum increase in testes mass during the pre-nesting period when I_G peaks, followed by a decrease in I_G during the nesting cycle until the N3 stage when I_G levels are lowest and no different from that of non-reproductive type I males. Despite finding no differences in I_G among type I males collected during the three stages of the nest cycle (N1–N3), histological examination of testes revealed that type I from N1 were full of mature sperm, while males from N2 and N3 had spermocytes and spermatids in addition to a high incidence of mature sperm. Thus, the presence of mature sperm along with other stages of spermatogenesis during the intermediate N2 and late N3 stages of the nest cycle in type I males is consistent with their ability for continued reproductive activity and protracted spawning over the course of the summer breeding season.

This study also indicates a seasonal cycle of sonic-muscle development in type I males that begins during the pre-nesting period prior to maximum I_{SM} levels that are maintained throughout the summer breeding season, followed by a decrease in I_{SM} during the non-reproductive winter when sonic muscles are regressed to minimum I_{SM} levels. Similar changes in I_{SM} are reported for the haddock *Melanogrammus aeglefinus* (L.), weakfish *Cynoscion regalis* (Bloch & Schneider) and *H. didactylus* in which sonic muscles hypertrophy for the reproductive spawning season and then atrophy afterwards during the non-reproductive season (Templeman & Hodder, 1958; Connaughton & Taylor, 1994; Modesto & Canario, 2003). The seasonal changes of I_{SM} in *C. regalis* and *H. didactylus* are congruent with seasonal changes in androgen levels for those species (Connaughton & Taylor, 1995; Modesto & Canario, 2003) and are also consistent with the reported seasonal fluctuations of androgen levels in type I male *P. notatus* (Sisneros *et al.*, 2004b). The plasma androgen levels of 11-ketotestosterone and testosterone in type I male *P. notatus* are low during the winter non-reproductive period, gradually increase during the seasonal re-crudescence of the testes in the spring pre-nesting period and then peak at the beginning of the summer nesting period (Sisneros *et al.*, 2004b). A number of

studies support the anabolic role of 11-ketotestosterone in the induction of secondary sex characteristics (e.g. sonic muscle growth and humming behaviour) that are necessary for courtship in type I males (Brantley *et al.*, 1993). The sustained, elevated levels of 11-ketotestosterone in type I males during the summer nesting season (Sisneros *et al.*, 2004b) may contribute to either the maintenance of sonic-muscle size and the sonic behaviour itself. In contrast to *P. notatus*, Johnson *et al.* (2000) found no evidence for seasonal changes in I_{SM} or sonic motor neuron size in the closely related oyster toadfish *Opsanus tau* (L.) and concluded that non-steroidal factors must be involved in the development of the sonic neuromuscular system for *O. tau* (Fine *et al.*, 2004).

An important finding of this study is that type I male number of offspring produced (measured as the number of viable late-stage embryos found in the males' nests) was positively correlated with the body mass of type I males at the end of the nests cycle (N3) when males have broods with at least 75% well pigmented, late-stage embryos. The N3 stage of the nest cycle is the last stage of embryonic development before young detach from the rocky nests and become free-swimming juveniles. In addition, DeMartini (1988) previously showed that the spawning success (measured as the total number of embryos in nests regardless of embryonic stage) was strongly influenced by the nest substratum area and the body mass of the type I males. DeMartini (1988), however, showed that the body mass of nesting males was a much better indicator of spawning success than the nest substratum area for type I males with natural nests than males with artificial nests. Results also show that the body condition of type I males was positively correlated with body mass during N3 of the nest cycle. Thus, previous and current results indicate that size (*i.e.* M_B) strongly influences male reproductive success and number of offspring produced such that larger type I males have greater spawning success, can provide parental care for more young, and are in better condition during the nesting season than smaller type I males.

Acoustic signals that are used as advertisement calls often contain information that can be used by conspecifics to assess the potential mate quality of the caller (Bradbury & Vehrencamp, 1998), especially when call characteristics are energetically expensive such as in the advertisement call or 'hum' produced by the type I male *P. notatus*. If sustained calling capacity is correlated with overall physiological fitness of the caller, then the acoustic signals can become good condition-dependent indicators of mate quality that can then be used by the receiver to facilitate mate-choice decisions. A recent study showed that in a wild population of *P. notatus* the frequency sensitivity of the saccule, the main organ of hearing in the fish, change with female reproductive state, such that summer reproductive females become better suited than winter non-reproductive females to encode the higher harmonic components of male advertisement calls (Sisneros & Bass, 2003). This enhanced encoding of the dominant frequency components of male advertisement calls by females during the summer breeding season may represent an adaptation to enhance the detection of the male advertisement call and possibly facilitate the acquisition of auditory information needed for mate-choice decisions. Future studies that investigate the call characteristics of the male advertisement call and its relationship to male body size (mass) will provide important insight into whether the *P. notatus*

advertisement call is a good condition-dependent indicator of mate quality for this species.

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