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Evaluating gonadosomatic index as an estimator of reproductive condition in the invasive round goby, *Neogobius melanostomus*

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ABSTRACT

Using gonadosomatic index cut-off scores has become a standard protocol for selecting reproductive fish in studies on the reproductive biology of round goby, *Neogobius melanostomus*, a significant invader of the Laurentian Great Lakes, but the validity of this practice has not been validated with histological staging. The goal of the current study was to evaluate the effectiveness of using gonadosomatic index (GSI) cut-off scores to classify reproductive status in male and female round goby by documenting associations between GSI, sex steroids, and gonad development. Gonadal stage was determined in both sexes using hematoxylin and eosin histology. Plasma 11-ketotestosterone and testosterone were measured in males, and testosterone and 17β-estradiol were measured in females. Gonadosomatic index cut-off scores were effective in selecting spawning capable individuals at higher GSI values, but GSI values were limited in the ability to make further distinctions of gonadal stage and missed many spawning capable females. In females, testosterone levels were highest during vitellogenic growth and declined prior to ovulation. 17β-estradiol displayed a similar, but non-statistically significant pattern. Males with developing testes had higher levels of 11-ketotestosterone —but not testosterone —than reproductively immature males, although levels of these androgens were overall positively correlated in males. The findings indicate that the conventional GSI cut-off scores (1% in males, 8% in females) accurately assign spawning capable condition in both sexes; however, they may also exclude some spawning capable females with lower GSIs.

Introduction

The round goby, *Neogobius melanostomus* (Pallas 1814), is a significant invader in the Laurentian Great Lakes of North America and several regions in Europe that has become a threat to native aquatic ecosystems worldwide (Corkum et al., 2004; Kornis et al., 2012; Poos et al., 2010). Since its invasion, considerable research has been conducted to assess its impacts and population dynamics by evaluating factors such as age and growth patterns, habitat use, and reproductive potential (Charlebois et al., 1997). One aspect of the round goby management plan that has received less attention is the development of standardized methods for reproductive condition assessment. Although it may be impractical to include detailed reproductive data into routine population assessments, precise measurement of reproductive condition is essential for determining reproductive cohorts to use in population growth models (Lowerre-Barbieri et al., 2011).

A variety of methods are available to assess reproductive condition in fishes, including microscopic gonadal staging, macroscopic gonadal staging, oocyte size–frequency distributions, sex steroid measurement and gonadal indices (Lowerre-Barbieri et al., 2011; West, 1990). Histological gonadal staging provides a direct snapshot of developmental stages of individual gametes within the gonads, while the relationship between other metrics and gonadal gamete stage(s) may depend on the pattern of gonadal development (e.g. synchronous vs. group synchronous vs. asynchronous). However, histological staging is time intensive and impractical in many cases, thus proxies are useful if they can be properly validated (Lowerre-Barbieri et al., 2011; West, 1990).

The gonadosomatic index (GSI = gonad mass / body mass × 100%, or GSI = gonad mass / somatic mass × 100%) (deVlaming et al., 1982) is a common metric of reproductive allocation and reproductive condition in fisheries biology. However, GSI may be an imprecise proxy for gonadal stage because it assumes isometry between somatic mass and gonad mass as well as a tight correspondence between total gonad mass and gonad developmental stage (deVlaming et al., 1982; Packard and Boardman, 1988). Nonetheless, if significant gonad growth occurs at distinct developmental stages (e.g. vitellogenesis), GSI cut-off scores (i.e. designating those above the score as 'reproductive' or 'spawning-capable') may be useful metrics for sorting fish into gross reproductive categories.

Gonadosomatic cut-off scores of 8% (females) and 1%–1.3% (males) are commonly used to select reproductive individuals in round goby invasion studies (Bowley et al., 2010; Gammon et al., 2005; Kasurak et al., 2012; Marentette et al., 2009; Marentette and Corkum, 2008; Yavno and Corkum, 2011; Young et al., 2010). Despite the widespread use of the GSI cut-off scores in such studies, there are scarce data available to

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validate this practice; neither are there much data on the correspondence between sex hormone levels and gonadal stage (but see Bowley et al., 2010). The goal of the current study was to examine the validity of GSI cut-off scores as reproductive condition classifiers in male and female round goby by examining the correspondence between GSI values, gonadal stage, and sex hormones. In this manner we provide the first direct test of association between these separate measures of reproductive condition in the round goby.

Materials and methods

Subjects

Round gobies were collected by angling from the shoreline of the Detroit River in Windsor, Ontario, Canada (42°18′23.83″N, 83° 4′32.46″W) between April and August 2011. 'Reproductive' males and females ('reproductive' criteria described below) were processed within 1 week of capture, whereas 'non-reproductive' fish were euthanized after being held in the laboratory for various lengths of time (see Supplementary Fig. 1 for GSI and hormone levels of reproductive fish in relation to ordinal date of processing). Gobies were kept in mixed sex tanks, fed with fish flakes (Tetramin Inc, Blacksburg, VA, USA), and kept on a 16L:8D photoperiod. All animal care and experimental protocols complied with the University of Windsor's animal care committee and guidelines of the Canadian Council of Animal Care (CCAC).

Gonadosomatic index was calculated, following fish euthanasia by clove oil overdose (~60 mg L^{-1}), according to the following equation: (GSI = gonad mass / total body mass × 100%) (Gammon et al., 2005; Marentette and Corkum, 2008). Gonad and body mass were measured to the nearest 0.01 g (Scout Pro scale, SP202, Ohaus Corp., Pine Brook, NJ, USA), and male gonad mass included both the testes and seminal vesicles. Fish sex could be easily determined in adults by examining urogenital shape (Charlebois et al., 1997). In accordance with previous round goby studies, females with GSIs greater than or equal to 8% were initially considered 'reproductive' (RFs) and less than 8% 'non-reproductive' (NRFs). Males with GSIs greater than or equal to 1% were scored as 'reproductive' (RMs) and less than 1% as 'non-reproductive' (NRMs). Masses, total lengths, and GSI in each reproductive category are given in Table 1.

No 'sneaker' males (i.e. reproductive males employing a 'parasitic' spawning strategy; Marentette et al., 2009; Taborsky, 1998), were included in the current study. Papilla indices (PI = papilla length / total length × 100%) of NRMs were below that of 'sneaker' males described by Marentette et al., 2009 (current study: 3.07 ± 0.04 SEM; Marentette study: 6.71 ± 0.12).

Steroid assays

Male plasma was assayed for 11-ketotestosterone (11KT) and testosterone (T), and female plasma for 17β-estradiol (E2) and T. Following euthanasia, the caudal peduncle region was severed and blood was drawn from the caudal vein using heparinized capillary tubes. The blood was spun for 10 min at 14,500 rpm (Micro-Hematocrit Centrifuge, LWS-M24, LW Scientific, Lawrenceville, GA, USA) and stored at −80 °C. The typical range of plasma volumes was 5–40 μL. All plasma was collected between 13:00 and 18:00 h to address potential diel influences on hormone levels. Steroids were extracted once with diethyl ether prior to performing enzyme-linked immunosorbent assays (Cayman Chemical, Ann Arbor, MI, USA). All samples were run in triplicate with individuals randomly assigned to plates. Inter-assay variation, calculated from a single control triplicate across plates, was 41.8% (n = 3 plates), 29.1% (n = 4), and 11.1%, (n = 2) for 11KT, T, and E2, respectively. Intra-assay variation was 5.2% ± 8.1%, 5.2% ± 6.1%, and 11.1% ± 13.2% for 11KT, T, and E2, respectively.

Because limited plasma volumes precluded measurement of extraction recoveries on all samples, representative recoveries were determined for each GSI-based reproductive group using cold spike recoveries on plasma pools comprised of equal volumes from at least 10 individuals (Bowley et al., 2010; 10 RM, 12 NRM, 10 RF, 17 NRF). The extraction recoveries in each reproductive group were as follows: RM: T = 13%, 11KT = 18%; NRM: T = 49%, 11KT = 56%; RF: T = 90%, E2 = 95%; NRF: T = 31%, E2 = 115%. Although androgen recoveries clearly varied across the representative plasma pools, recoveries were similar between 11KT and T within both RM and NRM pools. The variation in extraction recoveries was addressed in two ways: (1) androgen values were adjusted omnibus to 100% according to the recovery value of the sample's reproductive group plasma pool, and (2) tests for differences in androgen levels across gonad stage were performed separately for 'non-reproductive' and 'reproductive' GSI groups.

Gonad histology

Following GSI measurements, gonadal tissue was fixed in 10% neutral buffered formalin (after Marentette et al., 2010) and transferred to 70% ethanol within 3 months for long-term storage before histological processing (2–8 months). Testes were dehydrated, cleared with xylene, embedded in paraffin wax (Fisher Scientific, www.fishersci.com), and sectioned at 5–7 μm using a microtome (Leica RM2125, RT, Leica Microsystems Nussioch GmbH, Nussloch, Germany). Ovaries were embedded in a glycol methacrylate resin immuno-bed kit (Model #17324, Polysciences, Inc., Warrington, PA) and sectioned at 10 μm. Large gonads were cut in half along the medial–lateral axis before embedding, and multiple sections were taken near the middle of each gonad to minimize potential variation in cell type composition due to section location. Testis and ovary sections were mounted on Superfrost Plus slides (VWR International, LLC, Radnor, PA) and placed on a slide warmer to adhere tissue to slides. Slides were stained with hematoxylin and eosin (Fisher Scientific, www.fishersci.com) and viewed under a light microscope (Leica DME, Buffalo, NY).

Gonad staging criteria followed Brown-Peterson et al. (2011), and were based on the most advanced germ cell stage present (Table 2). Representative light micrographs of ovaries and testes in each stage are shown in Figs. 1 and 2, respectively. We used coalescence of the ooplasm yolk globules as our marker of maturation. Because degree of yolk globule coalescence varies among species (Patiño and Sullivan, 2002), this marker must be validated on a species-specific basis. We found this marker to be valid for work in female round goby since (1) all ovulated oocytes possessed fused yolk globules, (2) there was an increase in GSI in association with yolk globule fusion, and (3) T declined in association with this fusion.

Statistical analyses

All data were examined for normality using Kolmogorov–Smirnoff tests, and both hormone and male GSI data were log-transformed to meet normality assumptions. As indicated earlier, tests for differences in androgen levels across gonad stage were performed separately for 'non-reproductive' and 'reproductive' GSI groups due to differences in extraction recovery results. Differences in hormone level and GSI across

Table 1
Number of fish from each GSI category used for testosterone (T), 11-ketotestosterone (11KT) and 17β-estradiol (E2) steroid hormone assays as well as gonad histology with corresponding GSI and body size metrics (all mean ± SEM).

	T	11KT	E2	Gonad histology	GSI (%)	Mass (g)	Total length (cm)
NRM	22	24	NA	21	0.15 ± 0.03	15.3 ± 1.79	10.8 ± 0.32
RM	18	19	NA	19	1.8 ± 0.10	20.4 ± 2.50	11.8 ± 0.38
NRF	22	NA	22	22	1.9 ± 0.27	6.4 ± 0.60	8.1 ± 0.18
RF	17	NA	19	21	12.9 ± 0.59	6.8 ± 0.38	8.1 ± 0.17

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Table 2
Criteria used for classifying reproductive stage using round goby gonads (based on criteria by Brown-Peterson et al., 2011).

Sex	Stage	Histological features
Females	Immature	Only oogonia and primary growth oocytes are present.
	Cortical alveolar	Cortical alveolar oocytes are present.
	Developing	Cortical alveolar and/or vitellogenic oocytes (Vtg1 and Vtg2) are present. Yolk granules in vitellogenic oocytes are small, not filling the entire cytoplasm.
	Spawning-capable	Yolk globules are densely packed throughout the oocyte (Vtg3). Ooplasm may be partially fused.
	Mature	Yolk globules fused throughout ooplasm.
Males	Immature	Only spermatogonia are present; no lumen developed within lobules.
	Developing	Spermatocytes present, lumen formation. Spermatids and spermatozoa may also be present in spermatocysts, but they are not yet released into lumen.
	Spawning-capable	Spermatozoa are present in the lumen of the spermatocytes and some spermatogenesis is occurring in germinal epithelium. *Subphases:* (a) continuous germinal epithelium throughout (CGE), (b) evidence of discontinuous germinal epithelium (DGE)[a]
	Regressing	Residual reserves of spermatozoa are present in the lumen or sperm duct. There is no active spermatogenesis.

[a] As development progresses, lobules become filled with increasing proportions of spermatozoa, which is concurrent with reduced spermatogenesis and germinal epithelium becoming discontinuous (Grier, 2002).

gonadal stages were tested using either ANOVAs (followed by Tukey post-hoc tests) or *t*-tests, depending on the number of gonadal stages involved in the analysis. Pearson correlations between total length and GSI and somatic mass and GSI were performed within each reproductive group and found to be non-significant so body size was not included in further statistical analyses. Total length was unrelated to GSI in females ($r = -0.05$, $P = 0.69$) and in males ($r = 0.10$, $P = 0.52$). Total length was unrelated to any hormone levels in females (NRF: T: $r = 0.26$, $P = 0.52$; E2: r – 0.18, $P = 0.43$; RF: T: $r = 0.35$, $P = 0.17$; E2 $r = 0.11$, $P = 0.66$) or males (NRM: T: $r = 0.23$, $P = 0.30$; 11KT: $r = 0.13$, $P = 0.52$; RM: T: $r = 0.29$, $P = 0.25$, 11KT: $r = 0.09$, $P = 0.71$). The regressing stage in males was excluded from statistical tests due to this group's small sample size ($n = 3$). Pearson correlations were performed to examine covariation between E2 and T in females, and 11KT and T in males.

Within each reproductive category, GSI was related to androgen status using Pearson correlations and to E2 using second order polynomial curvilinear regression. Curvilinear regression was used for the E2

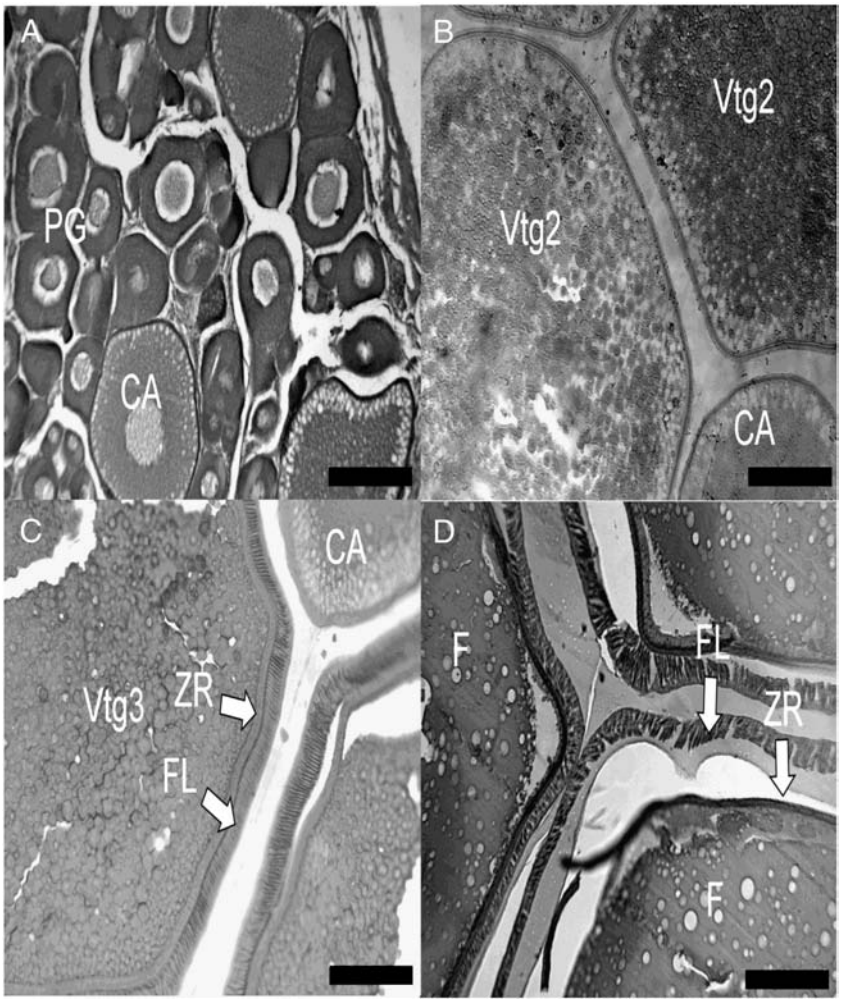

Fig. 1. Light micrographs of round goby ovaries in cortical alveolar (A), developing (B), spawning-capable (C), and mature (D) stages. Scale bars represent 200 μm. PG = primary growth oocyte, CA = cortical alveoli oocyte; Vtg2 = stage 2 vitellogenic oocyte, Vtg3 = stage 3 vitellogenic oocyte; ZR = zona radiata; FL = follicular layer; F = fused yolk globule oocyte.

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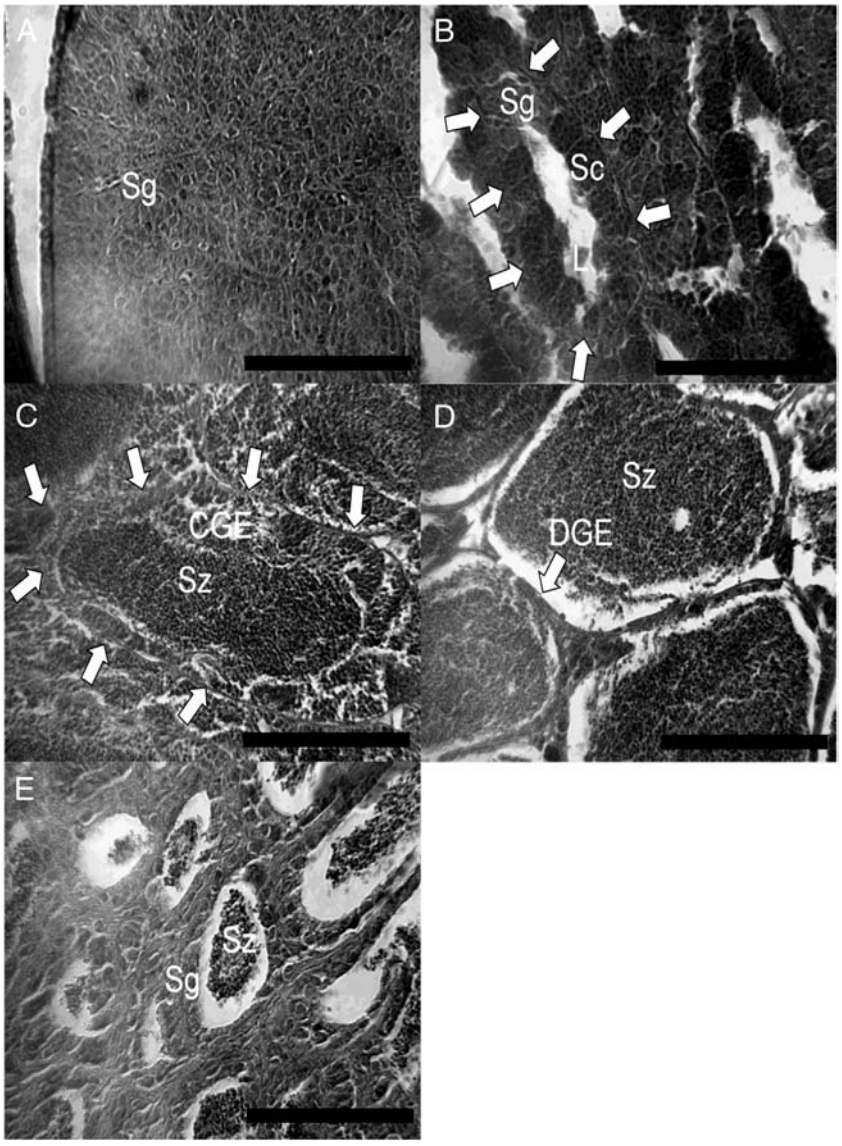

Fig. 2. Light micrographs of round goby testes in immature (A), developing (B), spawning-capable (C, D), and regressing (E) stages of development. Seminiferous lobules are outlined with arrows in panels B and C. Scale bars represent 100 μm. SG = spermatogonia, SC = spermatocytes, SZ = spermatozoa; L = lumen of lobule; CGE = germinal epithelium; DGE = discontinuous germinal epithelium.

analysis to test the expectation that E2 would increase to a peak during vitellogenesis and decline prior to ovulation (Devlin and Nagahama, 2002; Nagahama, 1994). Both 'reproductive' and 'non-reproductive' females were included in the curvilinear regression due to the similarity in E2 extraction recoveries between GSI classes. Overall hormone levels of 'reproductive' and 'non-reproductive' groups were compared using *t*-tests.

Results

Females

The 8% GSI cut-off score correctly assigned females as spawning capable and mature; however, some spawning capable females possessed GSIs below this value. Out of 22 NRF ovaries examined, eight were classified as cortical alveolar, 10 as developing, and four as spawning-capable. No immature ovaries (i.e. only oogonia present) were observed, and the vitellogenic egg cohort occurred alongside primary growth and cortical alveolar oocytes (Fig. 1). Out of 21 RFs examined, 10 possessed spawning-capable oocytes and 11 possessed mature oocytes. All except one ovary exhibiting ooplasm yolk globule coalescence were ovulating (i.e. follicular layer loose or ruptured, Fig. 1D).

There were significant differences in GSI across female reproductive stages, generally increasing with advancing stage ($F_{3,39} = 53.69$, $P < 0.001$) (Fig. 3A). Post-hoc tests indicated that GSI did not differ between cortical alveolar and developing stages, but the mature stage was marked by higher GSIs than the spawning-capable stage (Fig. 3A). There was no relationship between GSI and E2 ($R^2 = 0.09$, $P = 0.16$) (Fig. 3B), but GSI was positively associated with T in NRFs ($r = 0.57$, $P = 0.005$) and negatively associated with T in RFs ($r = -0.63$, $P = 0.006$) (Fig. 3C). 17β-estradiol and T were positively correlated in females, both when all females were considered together ($r = 0.52$, $P = 0.003$) and when NRFs and RFs were examined separately (NRFs: $r = 0.75$, $P = 0.001$; RFs: $r = 0.54$, $P = 0.036$) (Fig. 3D).

There were no statistical differences in E2 level across gonadal stages ($F_{3,30} = 0.91$, $P = 0.45$), although lowest levels of E2 were observed in the pre-vitellogenic cortical alveolar stage (Fig. 4A). Testosterone varied across gonadal stage in NRFs ($F_{2,15} = 5.78$, $P = 0.014$), such

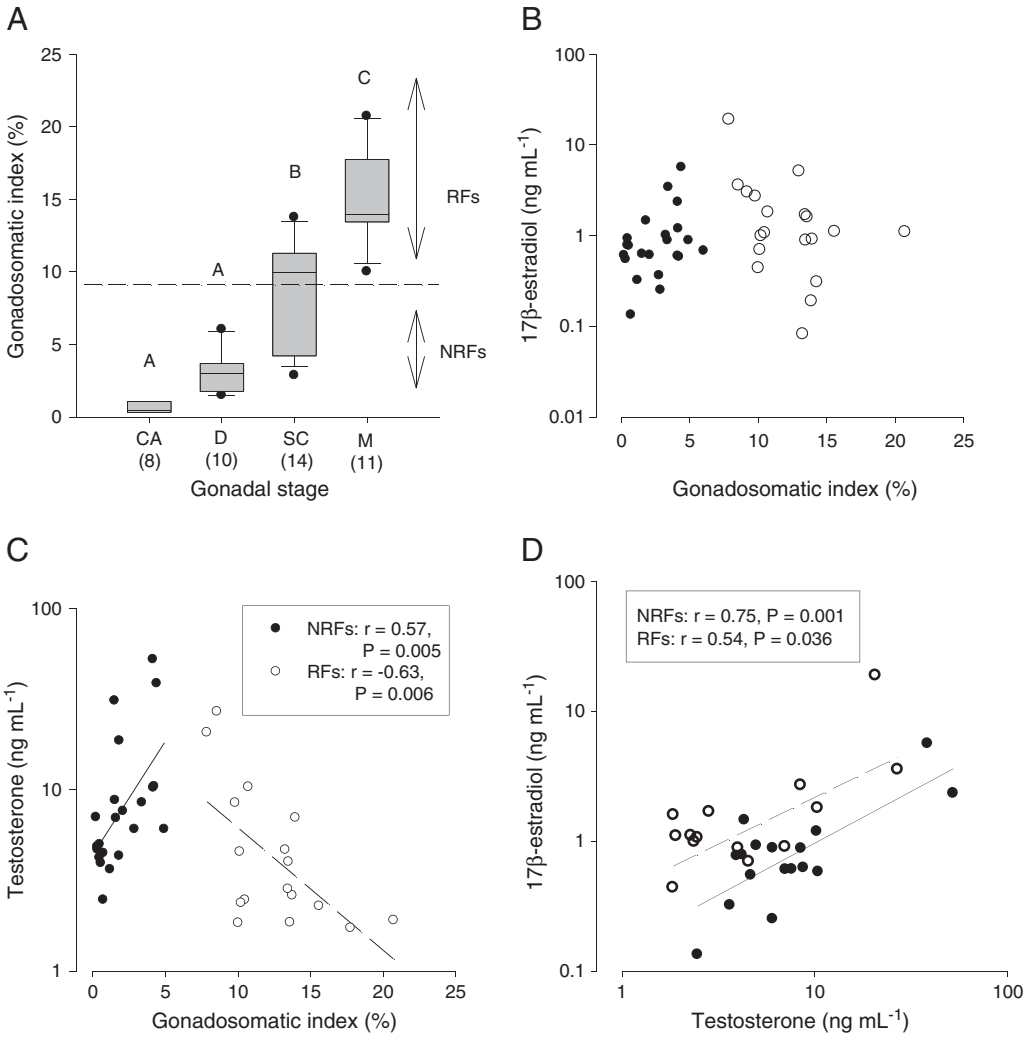

Fig. 3. Female GSI in relation to gonadal stage (A) and sex steroids (B, C). (A) Boxplot of GSI values across gonadal stages with a horizontal dashed line indicating the conventional 8% GSI cut-off and results of Tukey post-hoc tests indicated by letters above groups (CA = cortical alveolar, D = developing, SC = spawning-capable, M = mature). Whiskers of the boxplots represent 5th and 95th percentiles, and sample sizes are indicated in parentheses below each stage. In panel C, separate Pearson correlation analyses were performed separately for NRFs and RFs, and testosterone data were adjusted to 100% according to cold spike recovery results (see *Steroid assays* section for details). (D) Covariation between testosterone and 17β-estradiol in females.

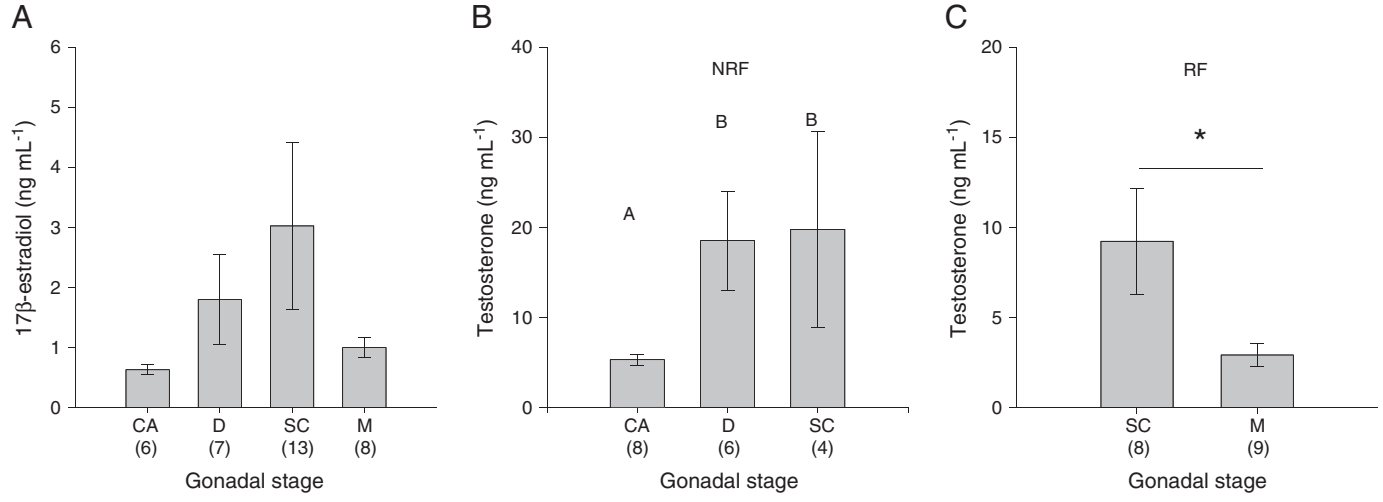

Fig. 4. Female 17β-estradiol (A) and testosterone (B, C) levels across gonadal stages. CA = cortical alveolar, D = developing (mid-vitellogenic), SC = spawning-capable (late vitellogenic), M = mature. Sample sizes are indicated below categories and error bars are standard errors. Testosterone values were adjusted to 100% based on reproductive group recoveries and levels across stages were examined separately for NRFs (B) and RFs (C). Letters over bars indicate significant differences in panel B while the asterisk and line indicate a significant *t*-test between groups in panel C.

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that developing and spawning-capable stages had higher levels than the cortical alveolar stage (developing: $P = 0.02$, late vitellogenic: $P = 0.059$) (Fig. 4B). In RFs, T was higher during the spawning-capable stage than the mature stage ($t_{12.5} = 2.54$, $P = 0.025$) (Fig. 4C). Between the GSI-based groups, T was higher in NRFs than in RFs (NRFs: 11.4 ± 12.8 ng mL^{-1}; RFs: 6.3 ± 7.2 ng mL^{-1}; $t_{37} = 2.37$, $P = 0.023$), and E2 was similar between NRFs and RFs (NRFs: 1.1 ± 1.3 ng mL^{-1}; RFs: 2.4 ± 4.2 ng mL^{-1}; $t_{39} = 1.43$, $P = 0.17$).

Males

The 1% GSI cut-off effectively classified the spawning-capable condition in males when used in conjunction with the presence of secondary sexual characteristics. Out of 19 RMs examined, 18 were classified as spawning-capable and one was classified as developing; the single developing RM lacked the distinct black coloration and enlarged cheek secondary sex characters. Within spawning-capable males, six had lobules with continuous germinal epithelium and 12 had at least some lobules with discontinuous epithelium. Out of 21 NRMs, eight were immature, 10 were developing, and three were regressing (Fig. 2A, B, E). Male GSI varied across gonadal stages, generally increasing as development proceeded ($F_{4,28} = 43.71$, $P < 0.001$) (Fig. 5A). Both 11KT and T were uncorrelated with GSI in both NRMs (11KT: $r = 0.30$, $P = 0.248$, T: $r = 0.23$, $P = 0.42$) and RMs (11KT: $r = 0.2$, $P = 0.42$; T: $r = 0.02$, $P = 0.94$) (Fig. 5B, C). 11-Ketotestosterone and T levels were positively correlated, both when examined as a whole ($r = 0.73$, $P < 0.001$) and when examined within each GSI-based group (NRMs: $r = 0.52$, $P = 0.011$; RMs: $r = 0.64$, $P = 0.004$) (Fig. 5D).

11-Ketotestosterone was higher in the developing stage than the immature stage ($t_{17} = 2.17$, $P = 0.045$) (Fig. 6A), although T levels were similar between these stages ($t_{15} = 0.17$, $P = 0.9$, unequal variance t-test) (Fig. 6B). Although the single developing RM was included in the NRM analyses, running the analysis without this datum did not affect the significant difference in 11KT between immature and developing stages. The transition from continuous germinal epithelium to discontinuous germinal epithelium (Fig. 2C, D) was not marked by changes in 11KT or T (11KT: $t_{15} = 0.41$, $P = 0.69$; T: $P = 1.00$, Mann–Whitney U test) (Fig. 6B). Between GSI-based groups, 11KT was higher in RMs than in NRMs (RMs: 30.9 ± 7.6 ng mL^{-1}; NRMs: 6.7 ± 1.7 ng mL^{-1}; 11KT: $t_{42} = 6.12$, $P < 0.01$). Similarly, T levels were higher in RMs than in NRMs (RMs: 5.26 ± 0.96 ng mL^{-1}; NRMs: 2.3 ± 0.3 ng mL^{-1}, T: $t_{39} = 4.50$, $P < 0.01$).

Discussion

A growing list of round goby studies have relied on cut-offs of 8% in females and 1% or 1.3% in males to separate individuals in 'reproductive' and 'non-reproductive' condition (Belanger et al., 2004, 2007; Gammon et al., 2005; Kasurak et al., 2012; Yavno and Corkum, 2011; Young et al., 2010). Our results indicate that the 1% cut-off was effective in separating

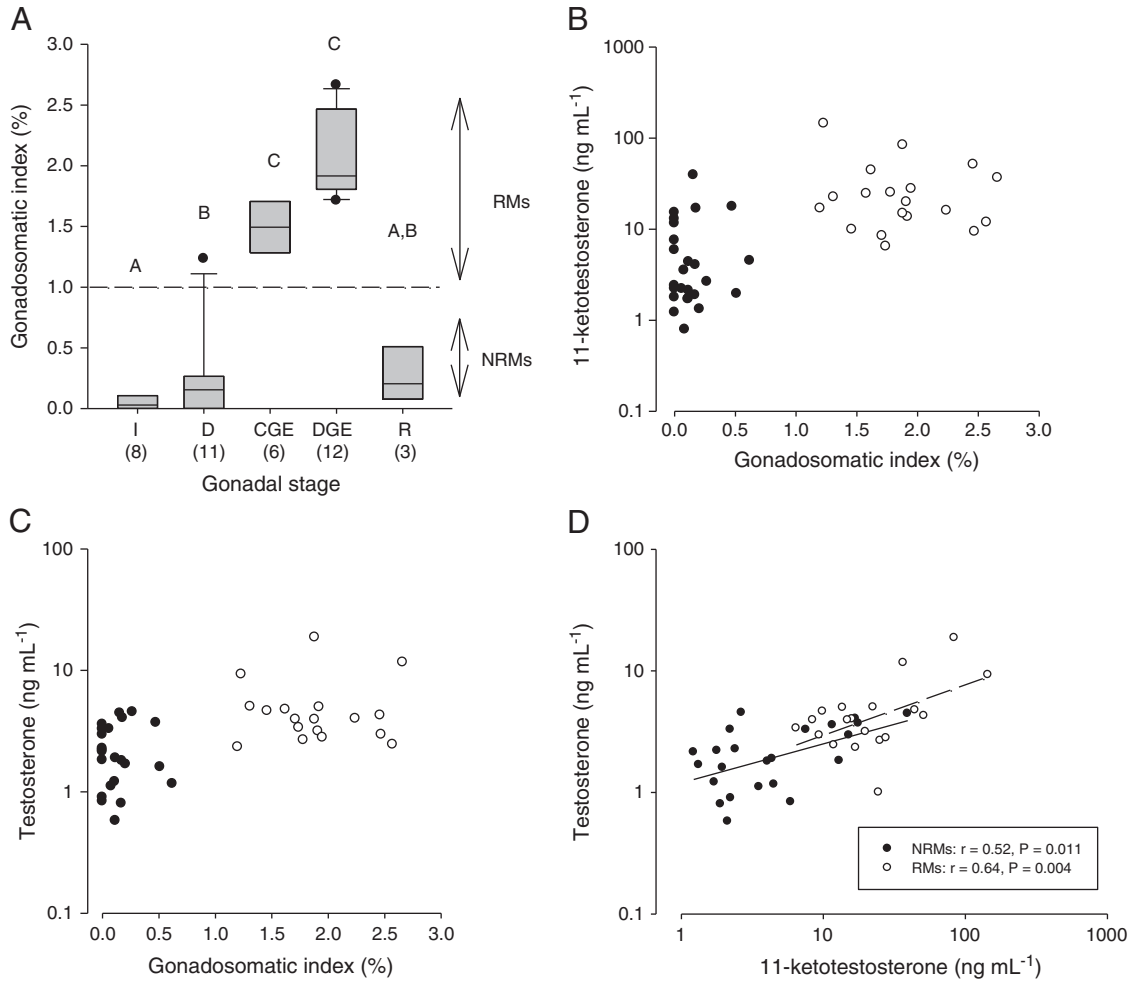

Fig. 5. Male GSI in relation to gonadal stage (A), 11-ketotestosterone (B), and testosterone (C). (A) Gonadosomatic index boxplots at progressing gonadal stages. I = immature, D = developing, CGE = continuous germinal epithelium, DGE = discontinuous germinal epithelium, R = regressing. Whiskers of the boxplots in panel A represent 5th and 95th percentiles. Sample sizes are indicated below each group. Horizontal line indicates the 1% cut-off, and results of Tukey post-hoc tests are indicated with letters. In panels B and C, NRMs are shown with filled circles and RMs are shown with open circles. (D) Covariation between testosterone and 11-ketotestosterone in males.

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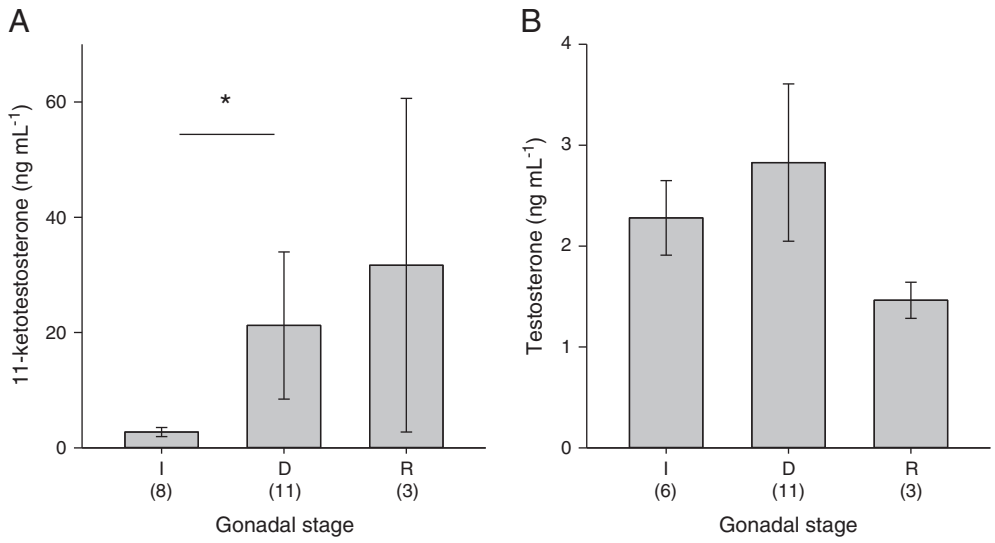

Fig. 6. Male 11-ketotestosterone (A) and testosterone (B) levels in non-spawning-capable stages (I = immature, D = developing, R = regressing). Sample sizes are indicated below each group. The asterisk and line indicate a significant *t*-test between groups.

spawning capable males from immature, developing, and regressing stage males when used in conjunction with secondary sexual characters. Females with GSI greater than 8% can similarly be assumed to be spawning capable; however, spawning-capable females occasionally possessed GSI values below 8% (~4%–5%), thus the 8% cut-off commonly used will result in an underestimate of spawning-capable females in a population.

While GSI generally increased with increasing gonadal stage in both sexes, this measure is of limited utility in delineating gonadal stages beyond the use as a cut-off score. In females, cortical alveolar stages and developing stages were overlapping in GSI values. In males, the 'NRM' category masks important distinctions between immature, developing, and regressed males. Although developing, spawning capable and mature stages were statistically significantly different in GSI, the overlap in GSI between these stages makes it impractical to use cut-off scores to distinguish these stages characterized by lower levels of gonad growth. Within spawning capable males, a trend for higher GSI in discontinuous germinal epithelium was evident, although not statistically significant due to a small sample size in the continuous germinal epithelium condition.

Gonadal steroids

One consideration that arises while analyzing our hormone data is that androgen extraction recoveries were sub-optimal and varied between reproductive plasma pools. Despite this variability, we feel that our recoveries did not affect the general patterns seen in the current study for multiple reasons: (1) androgen fluctuations in relation to gonadal development match biological predictions for both sexes, (2) steroids were correlated in each sex (E2 and T in females, 11KT and T in males), and (3) absolute androgen levels were within the range reported by others for the same species (Bowley et al., 2010; Marentette et al., 2009).

In females, T showed patterns of elevation during vitellogenesis and declined prior to ovulation, a common pattern in teleosts (Devlin and Nagahama, 2002; Kime, 1993; Lubzens et al., 2010; Nagahama, 1994). Testosterone rose and declined over the course of the reproductive cycle, which suggests that this hormone is a more useful marker of round goby reproductive development than E2, which did not change significantly. In females of both single and multiple spawning fishes, androgen and estrogen production typically decline as the ovarian follicles shift toward producing maturation steroids (Nagahama, 1994). In multiple spawning fishes, E2 levels commonly do not fluctuate sharply across individual batches, and the constant E2 may play a role in continued stimulation of growth of the next egg cohort (Pankhurst, 2008; Rinchard et al., 1993). The presence of cortical alveolar oocytes —recently linked to E2 elevation in coho salmon (Oncorhynchus kisutch) (Campbell et al., 2006) —throughout round goby ovarian development stages suggests that this process may also be occurring in female round goby. Additionally, similar vitellogenin mRNA expression levels between NRF and RF round goby suggest steady vitellogenic growth across the ovarian cycle (Bowley et al., 2010).

In males, elevated 11KT was associated with spermatogenesis (i.e. increased from the immature to the developing phases), which is consistent with several reports of 11KT, rather than T, stimulating spermatogenesis in fishes (Borg, 1994; Cavaco et al., 1998, 2001; Miura et al., 1991; Schulz et al., 2010). The concurrent elevation of 11KT and T observed in the current study is another common pattern in male fishes (e.g. Butts et al., 2012; Sisneros et al., 2004). We found no evidence that androgen level was related to degree of gonadal maturation (i.e. development of discontinuous germinal epithelium) in spawning capable males although additional variables such as captivity stress cannot be completely ruled out as a source of variability.

Conclusions

The current study found that GSI cut-offs were effective in characterizing many reproductive fish but incorrectly classified some fish as "non-reproductive", especially females. Therefore, below the 8% and 1% cut-offs, GSI alone is not recommended for distinguishing between specific gonadal stages. The data provided in this study will provide a reference for better understanding round goby reproductive potential which will ultimately assist our understanding of its invasion ecology.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jglr.2013.12.004.

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