Acoustic monitoring indicates a positive relationship between calling frequency and spawning in captive spotted seatrout (*Cynoscion nebulosus*)

Eric W Montie, Matt Hoover, Chris Kehrer, Justin Yost, Karl Brenkert, Timothy O'Donnell, Mike R Denson

Background: Fish sound production is widespread throughout many families. Agonistic and courtship behaviors are the most common reasons for fish sound production. Yet, there is still some debate on how sound production and spawning are correlated in many soniferous fish species. In the present study, our aim was to determine if a quantitative relationship exists between calling and egg deposition in captive spotted seatrout (*Cynoscion nebulosus*). This type of data is essential if scientists and managers plan to use acoustic metrics to identify spawning aggregations over large spatial scales and monitor reproductive activity over annual and decadal timeframes.**Methods**: Wild caught spotted seatrout were held in three laboratory tanks equipped with long-term acoustic loggers (i.e., DSG-Oceans) to record underwater sound throughout an entire, simulated reproductive season. Acoustic monitoring occurred from April 13 to December 19, 2012 for Tank 1 and from April 13 to November 21, 2012 for Tanks 2 and 3. DSG-Oceans were scheduled to record sound for 2 min every 20 min. We enumerated the number of calls, calculated the received sound pressure level (SPL in dB re 1 µPa; between 50 and 2000 Hz) of each 2 min 'wav file', and counted the number of eggs every morning in each tank.**Results**: Spotted seatrout produced three distinct call types characterized as "drums", "grunts", and "staccatos". Spotted seatrout calling increased as the light cycle shifted from 13.5 to 14.5 h of light, and the temperature increased to 27.7°C. Calling began to decrease once the temperature fell below 27.7 °C, and the light cycle shifted to 12 h of light. These captive settings are similar to the amount of daylight and water temperatures observed during the summer, which is the primary spawning period of spotted seatrout. Spotted seatrout exhibited daily patterns of calling. Sound production began once the lights turned off, and calling reached maximum activity approximately 3 h later. Spawning occurred only on evenings in which spotted seatrout were calling. Significantly more calling and higher mean SPLs occurred on evenings in which spawning occurred as compared to evenings in which spawning did not occur. Spawning was more productive when spotted seatrout produced more calls. For all tanks, more calling and higher SPLs were associated with more eggs released by females.**Discussion**: The fact that more calling and higher SPLs

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were associated with spawns that were more productive indicates that acoustic metrics can provide quantitative information on spotted seatrout spawning in the wild. These findings will help us to identify spawning aggregations over large spatial scales and monitor the effects of noise pollution, water quality, and climatic changes on reproductive activity using acoustic technology.

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Acoustic Monitoring Indicates a Positive Relationship between Calling Frequency and Spawning in Captive Spotted Seatrout (*Cynoscion nebulosus*)

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Running Headline: Spotted seatrout calling and spawning

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ABSTRACT

Background: Fish sound production is widespread throughout many families. Agonistic and courtship behaviors are the most common reasons for fish sound production. Yet, there is still some debate on how sound production and spawning are correlated in many soniferous fish species. In the present study, our aim was to determine if a quantitative relationship exists between calling and egg deposition in captive spotted seatrout (*Cynoscion nebulosus*). This type of data is essential if scientists and managers plan to use acoustic metrics to identify spawning aggregations over large spatial scales and monitor reproductive activity over annual and decadal timeframes.

Methods: Wild caught spotted seatrout were held in three laboratory tanks equipped with long-term acoustic loggers (i.e., DSG-Oceans) to record underwater sound throughout an entire, simulated reproductive season. Acoustic monitoring occurred from April 13 to December 19, 2012 for Tank 1 and from April 13 to November 21, 2012 for Tanks 2 and 3. DSG-Oceans were scheduled to record sound for 2 min every 20 min. We enumerated the number of calls, calculated the received sound pressure level (SPL in dB re 1 µPa; between 50 and 2000 Hz) of each 2 min 'wav file', and counted the number of eggs every morning in each tank.

Results: Spotted seatrout produced three distinct call types characterized as "drums", "grunts", and "staccatos". Spotted seatrout calling increased as the light cycle shifted from 13.5 to 14.5 h of light, and the temperature increased to 27.7 °C. Calling began to decrease once the temperature fell below 27.7 °C, and the light cycle shifted to 12 h of light. These captive settings are similar to the amount of daylight and water temperatures observed during the summer, which is the primary spawning period of spotted seatrout. Spotted seatrout exhibited daily patterns of calling. Sound production began once the lights turned off, and calling reached maximum activity approximately 3 h later. Spawning occurred only on evenings in which spotted seatrout were calling. Significantly more calling and higher mean SPLs occurred on evenings in which spawning occurred as compared to evenings in which spawning did not occur. Spawning was more productive when spotted seatrout produced more calls. For all tanks, more calling and higher SPLs were associated with more eggs released by females.

Discussion: The fact that more calling and higher SPLs were associated with spawns that were more productive indicates that acoustic metrics can provide quantitative information on spotted seatrout spawning in the wild. These findings will help us to identify spawning aggregations over large spatial scales and monitor the effects of noise pollution, water quality, and climatic changes on reproductive activity using acoustic technology.

INTRODUCTION

The Family Sciaenidae contains fish renowned for their sound producing capabilities. These include such species as red drum (*Sciaenops ocellatus*), weakfish (*Cynoscion regalis*), American star drum (*Stellifer lanceolatus*), Atlantic croaker (*Micropogonias undulatus*), black drum (*Pogonias cromis*), silver perch (*Bairdiella chrysoura*), and spotted seatrout (*Cynoscion nebulosus*) (e.g., Hill et al., 1987; Nieland & Wilson, 1993; Sprague et al., 2000; Collins et al., 2001; Montie et al., 2015a). Sciaenids may have evolved mechanisms to produce sound in order to communicate in turbid estuarine, bay, and coastal systems (Holt et al., 1981; Moyle & Cech, 1988; Holt, 2008). Sound production morphology in this family involves a sonic muscle that abuts a swimbladder (reviewed in Fine & Parmentier, 2015). This muscle contracts near the inflated swim bladder and results in a drumming sound. In most Sciaenids, males are the prominent sound producers; however, in the Atlantic croaker and black drum, both male and female contain sonic muscles and produce sound (Hill et al., 1987; Tellechea et al., 2010). Agonistic and courtship behaviors are the most common reasons for fish sound production. However, there is still some debate on how sound production and spawning are correlated in many soniferous fish species (e.g., Luczkovich et al., 1999a; Locascio et al., 2012).

Studies that have recorded underwater sound during spawning seasons have revealed that patterns of fish sound production coincide with patterns of reproductive condition (Connaughton & Taylor, 1995). Other studies have shown an association between sound production and spawning in the wild through the simultaneous collection of acoustic recordings and eggs (Mok & Gilmore, 1983; Saucier & Baltz, 1993; Luczkovich et al., 1999a; Aalbers & Drawbridge, 2008). For example, in wild weakfish, Luczkovich et al. (1999a) found that the timing and levels of sound production were significantly correlated with the timing and numbers of “sciaenid-type" eggs. These types of comparisons are essential if scientists plan to use acoustic metrics as a tool to monitor fish reproduction. These data are challenging to obtain because it is difficult to ensure that the eggs that are collected are from the same population of fish that are producing sound (Locascio et al., 2012). In the field, it is likely that the number of eggs collected is affected by predator activity, water currents, and the efficiency of plankton tows.

Studies using fishes held in captive tanks can control for some unaccounted variables that are present in the wild. A few studies have used this captive approach to examine the associations of sound production and spawning (Guest & Lasswell, 1978; Connaughton & Taylor, 1996; Montie et al., 2015b). Connaughton and Taylor (1996) illustrated the association between courtship behavior, male drumming, and spawning in weakfish held in laboratory tanks. Recently, Montie et al. (2015) collected quantitative data to clarify the relationship between calling, call structure, and eggs produced in a captive population of red drum. Spawning occurred only on evenings in which red drum were calling, and spawning was more productive when red drum produced more calls with longer durations and more pulses.

In the present study, our overall goal was to collect quantitative data to understand the relationship between the amount of calling and the number of eggs collected in laboratory tanks containing spotted seatrout. We utilized acoustic loggers that recorded the underwater tank environment throughout an entire, simulated reproductive season. Spotted seatrout are an estuarine-dependent species ranging from Cape Cod, Massachusetts to Key West, Florida and from southwest Florida to southern Mexico in the Gulf of Campeche (Welsh & Breder, 1924; Mather, 1952; Tabb, 1966). The primary reproductive period occurs during early April through late October along the Atlantic coast and the Gulf of Mexico. Tabb (1966) was the first to show that male spotted seatrout produce a drumming sound associated with courtship and spawning.

Our specific objectives in the present study were to:(i) describe and characterize the calls of wild spotted seatrout held in captivity; (ii) determine if spotted seatrout exhibited daily patterns of sound production; (iii) investigate the relationship between the levels of sound production with the number of eggs collected; and (iv) determine if changes in call types or structure affected spawning productivity.

MATERIALS AND METHODS

Sexually mature spotted seatrout were captured from the wild and placed into captivity by researchers at the South Carolina Department of Natural Resources (SCDNR) in Charleston, South Carolina (Table 1). Fish were fed equal parts of Boston mackerel (*Scomber scombrus*), squid, and shrimp three times a week. Spotted seatrout were held in three separate, 3.67 m diameter fiberglass tanks (i.e., Tank 1, Tank 2, and Tank 3) with individual recirculating aquaculture systems equipped with UV sterilizers, protein fractionators, and bead filters. These indoor tanks were circular, 1.7 m deep, and were filled with settled, sterilized Charleston Harbor seawater. Tank temperatures were individually controlled. Water temperature and diurnal periodicity were maintained at a predetermined cycle that followed scheduled photoperiod and temperature adjustments that resembled a natural reproductive season and encouraged spawning. However, in Tank 1, the temperature was maintained at 27.8°C and the photoperiod was left at 14.5 h light until late November to maintain spawning activity. Acoustic monitoring occurred from April 13 to December 19, 2012 for Tank 1 and from April 13 to November 21, 2012 for Tanks 2 and 3. Floating eggs were collected from a surface, skimming port in the side of the tank that drained into an egg collection tank equipped with a 250-micron mesh net. Collection nets were checked each morning for the presence of eggs. If eggs were present, they were collected and placed in a 15 L *Artemia* hatching cone for separation into floating (i.e., fertile) and sinking (i.e., unfertilized) eggs. Once separated, eggs were drained into graduated cylinders where they were enumerated volumetrically. A subsample of 200 floating eggs and sinking eggs were evaluated using microscopy to determine if embryos were present. Floating eggs were then transferred to 500 L incubation cones until hatching occurred.

We deployed long-term acoustic recorders (DSG-Oceans, Loggerhead Instruments, Sarasota, FL, USA, www.loggerhead.com) into each tank prior to the onset of spawning activity following methods previously described (Montie et al., 2015b). Briefly, the DSG-Ocean is composed of a cylindrical PVC housing with a High Tech Inc. hydrophone (-185 dBV μPa^{-1}) attached to a microcomputer circuit board and powered by 24 D-cell alkaline batteries. The DSG board incorporates an additional 20 dB of gain and is calibrated with a 0.1 V (peak) frequency sweep from 2 – 100 kHz. For this experiment, DSG-Oceans were set to a sampling rate of 50 kHz and contained a 35 kHz 3-pole low-pass filter on the hydrophone input. DSG-Oceans were scheduled to record sound for 2 minutes every 20 minutes (e.g., 12:00 to 12:02; 12:20 to 12:22; 12:40 to 12:42, 13:00 to 13:02, etc.) and saved as 'DSG files' on a 128 GB SD-card. Recorders were retrieved a total of nine times during the experiment, once on May 5th , May 7th , May 25th, June 25th , July 23rd, August 24th, September 21st, and October 26th to change batteries and download the recorded files and again on November 21st and December 19th to download final data. In Tank 1, acoustic recordings were not collected between 16:40, May 18th and 19:40, May 22nd, 2012 due to recorder malfunction. The 'DSG files' were transferred to a hard drive and batch converted into 'wav files' using DSG2wav© software (Loggerhead Instruments, Sarasota, FL, USA).

We manually counted the number of calls within each 2 min, ‘wav file’ by viewing the files in Adobe Audition (Adobe Systems Incorporated, San Jose, CA, USA, www.adobe.com). Spotted seatrout produced three different calls, which we characterized as “grunts”, “drums”, or “staccatos” following similar nomenclature previously published in other studies (Mok & Gilmore, 1983; Sprague et al., 2000; Walters et al., 2009). For each tank, we determined the number of “grunts”, “drums”, and “staccatos” per day by summing the calls that occurred between 18:00 and 06:00, which was the time in which the majority of calling occurred.

We calculated the received sound pressure level (SPL; dB re 1 uPa; between 50 and 2000 Hz) of the entire, 2 min ‘wav file’ using automated MATLAB scripts (The MathWorks, Inc., Natick, MA, USA, www.mathworks.com). Received SPL calculations were completed by first applying a band pass filter to the signal, then calculating the root-mean-square (RMS) voltage, and then converting the RMS voltage to a received SPL by incorporating the hydrophone sensitivity (-185 dBV μPa^{-1}) and the DSG gain (i.e., 20). Files that contained noise artifacts created from tank filters, tank maintenance, and fish hitting the tank walls or recorders were not included in SPL analysis. The average background noise levels for Tanks 1 through 3 were approximately 114, 113, and 112 dB re 1 µPa, respectively. The highest received SPLs for tanks 1 through 3 were 148 (i.e., 139 calls detected), 146 (i.e., 76 calls detected), and 138 (i.e., 67 calls detected) dB re 1 µPa, respectively. For each tank, we calculated the mean SPL per day by taking the mean of the SPLs between 18:00 and 06:00.

For each day during the recording period, the ‘wav’ file that contained the most numerous calls was used to estimate the mean duration and mean number of pulses in a “staccato” call for that day. We calculated “staccato” duration by manually subtracting the time of call termination from the time of call initiation. The pulse number was determined by manually counting each individual pulse in a “staccato”.

Microsoft Excel (Microsoft, Redmond, WA, USA, www.microsoft.com/en-us), MATLAB, and SYSTAT 13 (Systat Software, Inc., San Jose, CA, USA, www.systat.com) were used for data and statistical analysis. The time and frequency domains were illustrated for “drums”, “grunts”, and “staccatos”. We summarized spotted seatrout spawning productivity, the number of calls and SPLs, and call characteristics for each tank. We determined the relationship between all calls (i.e., sum of “grunts”, “drums”, and “staccatos”) and received SPLs for each tank using Pearson correlation analysis. We plotted the number of calls, water temperature, and photoperiod adjustments versus date. To examine the daily patterns of sound production in each tank, we determined the mean number of “drums” for each time interval during the 14.5 h of light photoperiod.

We examined the relationship between sound production and spawning. To determine if spawning was associated with spotted seatrout calling, we plotted the number of calls per day and the number of eggs collected (i.e., the next morning) versus the date for each tank. To examine whether or not more calling (i.e., the number of calls between 18:00 and 06:00) and higher SPLs (i.e., the mean SPL between 18:00 and 06:00) occurred on evenings with spawning, we performed paired T-tests. We performed linear regressions with the number of calls or mean SPL per evening as the independent variable and the number of eggs collected as the dependent variable. To examine whether or not calls of longer duration with more pulses occurred on the evenings with spawning, we performed paired T-tests.

RESULTS

Acoustic Characterization of Spotted Seatrout Calls

Spotted seatrout held in captivity produced three distinct call types. These calls were characterized as “drums”, “grunts”, or “staccatos”, following similar nomenclature published in other studies (Fig. 1; Mok & Gilmore, 1983; Sprague et al., 2000; Walters et al., 2009). The acoustic energy of the calls occurred between 50 and 1500 Hz with most energy occurring between 50 and 500 Hz (Fig. 1E-G). A staccato call was characterized as having multiple pulses ($n > 5$) with a very short inter-pulse interval with acoustic energy ranging from 50 to 1000 Hz (Fig. 1A, B, E). A grunt call was composed of a single pulse displaying multiple harmonics with acoustic energy ranging from 50 to 1000 Hz (Fig. 1A, C, F). A drum call was composed of one to four pulses with a short inter-pulse interval with acoustic energy ranging from 50 to 1000 Hz (Fig. 1A, D, G). The number of drums, grunts, and staccatos were positively correlated with each other in all tanks (Pearson Correlation Test; $P < 0.05$ for all comparisons, data not shown). In all tanks, drums were the most frequently produced followed by grunts and then staccatos (Table 1; Fig. 2). Increased calling led to higher average SPLs, and the total number of calls (i.e., sum of drums, grunts, and staccatos) produced correlated positively with SPL in all tanks (Pearson Correlation Test; $r = 0.917$, $P < 0.01$ for Tank 1; $r = 0.688$, $P < 0.01$ for Tank 2; and $r = 0.457$, $P < 0.01$ for Tank 3).

Patterns of Sound Production

We observed three major findings concerning general patterns of sound production. First, fish calling occurred in all tanks (Table 1; Fig. 2). Second, the amount of calling differed among tanks (Tank 1 > Tank 2 > Tank 3; Table 1; Fig. 2). Third, photoperiod and temperature adjustments affected calling. As the simulated reproductive season progressed, maximal sound production occurred when the photoperiod shifted to 14.5 h of light, and the water temperature increased to 27.7°C; calling began to decrease once the temperature fell below 27.7 °C, and the light cycle changed to 12 h light per day. In Tank 3, which showed the least amount of calling and had the lowest number of males, spotted seatrout calling was more sporadic (Fig. 2C). The general pattern was that calling increased as the light cycle shifted from 12.5 to 14.5 h light per day and as the temperature increased to 27.8 °C. Between 9/21/2012 and 11/8/2012, calling was more prevalent. Calling began to decrease once the temperature fell below 24.0 °C on 11/14/2012. In all tanks, abrupt drops in temperature decreased calling, while abrupt rises in temperature increased sound production (Fig. 2).

Spotted seatrout exhibited daily patterns of calling in all tanks (Fig. 3). Generally, sound production began once the lights turned off (i.e., 17:45). The highest number of drums occurred at 21:20 in Tank 1; 21:00 in Tank 2; and 20:40 in Tank 3(Fig. 3). The number of grunts, staccatos, and received SPLs followed similar patterns (data not shown).

Relationship between Sound Production and Spawning

Calling played an important role in spawning of wild caught spotted seatrout held in captivity. This overall theme was supported by four major findings. First, we discovered that successful spawns (i.e., eggs were present) occurred only on evenings in which spotted seatrout were calling (Fig. 4). Spotted seatrout did produce sound without a corresponding spawn, but spawning never occurred without a substantial increase in calling the evening before eggs were collected. Second, we found that significantly more calling and higher mean SPLs occurred on evenings in which spawning occurred as compared to evenings in which spawning did not occur (Table 2). Third, we demonstrated that spawning was more productive with more calling. For all tanks, more calling and higher SPLs were associated with more eggs released by females (Table 3). Fourth, we discovered that tanks with more sound production had more spawns (Tank 1 > Tank 2 > Tank 3; Table 1). Tanks with more calling and higher mean SPLs calculated over the entire monitoring period resulted in larger total egg yields per gram female biomass (Fig. 5). We did test whether or not the number of pulses and the total duration of a staccato were different on evenings when spawning did occur as compared to evenings when spawning did not occur, but we found no significant difference (Table 2).

DISCUSSION

Acoustic Characterization of Spotted Seatrout Calls

Our characterization of spotted seatrout calls were similar to the findings observed in other research studies (Mok & Gilmore, 1983; Luczkovich et al., 1999b; Sprague et al., 2000; Luczkovich et al., 2008). Mok & Gilmore (1983) recorded and classified spotted seatrout sounds into four call types: i) "grunt followed by knocks"; ii) "aggregated grunts"; iii) "long grunt"; and iv) "staccato". In our captive study, we grouped the "grunt followed by knocks" and "aggregated grunts" together because of their similarity in call structure and described these calls as "drums" to avoid confusion with the "grunt". We classified the "long grunt" as the "grunt", while the "staccato" strictly followed the description provided by Mok & Gilmore (1983). Mok & Gilmore (1983) found that the "grunt followed by knocks" occurred within a frequency range from 300 to 1350 Hz, while most of the acoustic energy of the "aggregated grunts" was distributed from 220 to 600 Hz. The "long grunt" described by Mok & Gilmore (1983) contained several harmonic bands from 200 to 1400 Hz, which was similar to the "grunt" we described in the present study. The "staccato" described by Mok & Gilmore (1983) consisted of a series of pulses with the

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acoustic energy distributed from 200 to 600 Hz, which was similar to the “staccato” we described in the present study.

We calculated the received SPL between 50 and 2000 Hz of the entire 2 min ‘wav’ file. Sound pressure level is the most universal acoustic metric and expresses RMS sound amplitude within a given time window (in this case 2 min) and frequency range (in this case 50 to 2000 Hz) as a single decibel level (Kinsler et al. 1999). We demonstrated that the total number of calls (i.e., sum of drums, grunts, and staccatos) counted in the 2 min ‘wav’ files correlated positively with the mean received SPL. Mean SPL is a function of the number of calls, duration of each call, the sound intensity of each call, and the distance of the sound source from the recorder. The relationship between calling and received SPL is important because SPL is often the more useful metric in quantifying sound production in the wild, where it is not possible to count overlapping calls of a chorusing aggregation. In addition, long-term monitoring of spawning aggregations using autonomous acoustic recorders can generate several thousand acoustic files. Having a MATLAB code to determine the mean received SPL of each acoustic file as a means to quantify sound production is much less time intensive than having an observer manually count calls. The one drawback in calculating received SPL is that the level depends on the distance from the spawning aggregation, which is typically unknown in wild recordings.

Light Cycle and Temperature Affect Sound Production

We discovered that maximal sound production of captive spotted seatrout occurred when the photoperiod shifted to 14.5 hrs of light, and the water temperature increased to approximately 27°C. These captive settings are similar to the amount of daylight and water temperatures observed during the summer in the Southeast, which is the primary spawning period for spotted seatrout (Luczkovich et al., 1999b; Roumillat and Brouwer, 2004; Luczkovich et al., 2008). In the southeastern United States, sound production of spotted seatrout has been detected from May to September (Riekerk, Tyree, & Roumillat, 1997; Luczkovich et al., 2008). This seasonal shift in sound production is most likely due to changes in circulating testosterone levels, which affects the output of the brain and sonic muscle mass. For example, as the spawning season approaches, the sonic muscle in weakfish triples in mass, which coincides with seasonal patterns of peak calling (Connaughton & Taylor 1994; Connaughton & Taylor, 1995). This hypertrophy is driven by elevated androgen levels, which are triggered by photoperiod and temperature cues that initiate sexual behavior (Connaughton & Taylor, 1994). Wild and captive spotted seatrout may follow similar endocrine, anatomical, and physiological changes.

We found that wild caught spotted seatrout exhibited daily patterns of calling, which began when the lights turned off and reached maximum activity three hours later. Other sciaenid species held in captivity, including Atlantic croaker, sand seatrout (*Cynoscion arenarius*), and red drum, have been shown to exhibit similar daily patterns of spawning, with reproductive activity arising during or soon after laboratory-simulated dusk (Holt et al., 1985; Montie et al., 2015b). In Charleston Harbor, South Carolina, spotted seatrout calling was shown to occur from 18:00 to 22:00 hs, with peaks occurring in the late evening (Riekerk, Tyree, & Roumillat, 1997). In Pamlico Sound, North Carolina, spotted seatrout drumming activity began after sunset (21:00 hs), peaked at 22:00 hs, and ended at 23:00 hs (Luczkovich et al., 2008). In Barataria, Caminada, and Eastern Timbalier Bay Systems of Louisiana, Saucier and Baltz (1993) showed that spotted seatrout sound production occurred from 17:00 to 01:00 hs and that 92% of the drumming occurred between 19:00 and 23:00 hs.

Spawning at dusk is a reproductive strategy for sciaenids living in a temperate environment (Holt et al., 1985). This evolved behavior may provide a fitness advantage for offspring by limiting predation on eggs (Holt et al., 1985). Many fishes including those of the families Crangidae, Lutjanidae, and Sciaenidae prey on recently released sciaenid eggs. It is suggested by Holt et al. (1985) that predation by these fishes occurs mostly during the day because these predators lack the sensory abilities to detect prey during the night. Thus, evening spawning allows for maximum dispersal of eggs prior to dawn when predators become more active. In fact, Holt et al. (1985) performed plankton tows and found that spotted seatrout egg densities were reduced from 100 m^{-3} during spawning to less than 1 m^{-3} the next afternoon, after 24 h of wind and tide dispersal.

As previously discussed, maximal sound production of captive spotted seatrout occurred when the photoperiod shifted from 13.5 to 14.5 h of light, and the water temperature increased to approximately 28^{o}C, which simulated daylight and water temperatures observed during the summer spawning season in South Carolina (Fig. 2). These photoperiod and temperature cues initiate the spawning season of spotted seatrout. However, in all tanks, rapid temperature changes within the simulated reproductive season, also affected calling (Fig. 2). Generally, abrupt drops in temperature decreased calling, while abrupt rises in temperature increased sound production. In a different but similar study, we found that wild caught red drum held in laboratory tanks exhibited similar temperature dependent behaviors (Montie et al., 2015b). Other studies with different fish species revealed similar findings. Schneider (1967) illustrated that the number of calls and the number of pulses in a call produced by tiger bass (*Terapon jarbua*) increased at higher temperatures. Fine (1978) found that elevated water temperatures increased the fundamental frequency and occurrence of mating calls produced by wild oyster toadfish

(*Opsanus tau*). Connaughton et al. (2000) found that a rise in water temperature increased the mean SPL, the mean number of pulses, and the mean frequency in Hz of captive weakfish calls. Maruska and Mensinger (2009) reported that higher water temperatures were correlated with a greater number of grunt emissions, higher fundamental frequencies, and shorter call durations in oyster toadfish.

Sound Production Influences Spawning Success

In this study, we provide quantitative data on the positive relationship between sound production and spawning in captive spotted seatrout. In a different but similar study, we found that the amount of calling and the call structure played an important role in spawning success in wild caught red drum held in laboratory tanks (Montie et al., 2015b). Other studies performed in captive environments with red drum, weakfish, and white seabass (*Atractoscion nobilis*) have demonstrated a qualitative association between sound production and spawning (Guest and Laswell, 1978; Connaughton and Taylor, 1996; Lowerre-Barbieri et al., 2008; Aalbers and Drawbridge, 2008).

In a wild setting, floating eggs and juveniles of many sciaenids have been collected with plankton tows on the same night and at the same location in which species specific calls have been recorded, signifying an association between sound production and spawning (Mok & Gilmore, 1983; Saucier & Baltz, 1993; Connaughton & Taylor, 1995; Luczkovich et al., 1999a). Mok & Gilmore (1983) revealed that peak calling of black drum, silver perch, and spotted seatrout occurred between 17:00 and 22:00 hours in the Indian River Lagoon, Florida and that these maximums coincided with the appearance of eggs and larvae in the water column. Saucier and Baltz (1993) collected spotted seatrout eggs in the Gulf of Mexico using plankton tows and showed that tows downstream of drumming aggregations contained two to three times more eggs in comparison to tows upstream. Luczkovich et al. (1999a) found that SPLs of weakfish and silver perch aggregations at stations in Pamlico Sound, North Carolina positively correlated with “sciaenid-type” egg densities. However, Locascio et al. (2012) reported that the timing and levels of sound production were negatively associated with those of egg production in black drum inhabiting an estuarine canal basin of Cape Coral, Florida. These findings were unexpected and may be explained by the possible differences in the spawning potential of the female population in the study area over the period in which sampling occurred.

Our findings did not indicate that the call type (i.e., drums, grunts, or staccatos) or call structure (i.e., the number of pulses and total duration of the staccato) played a differential role in spawning success. However, in a different but similar study, we found that the call structure did play an important role in spawning success in wild caught red drum held in laboratory tanks (Montie et al., 2015b). In that study, we demonstrated that the mean number of pulses in a call was higher and the mean call duration was longer on evenings when spawning did occur as compared to evenings when spawning did not occur (Montie et al., 2015b). We provided ample evidence that sound production equates to spawning in captive red drum, when calls were longer than 0.8 seconds and contained more than seven pulses. This difference between spotted seatrout and red drum may be a factor of the duration of their respective spawning seasons. The spawning season for red drum is much shorter (i.e., August to October) than the season observed in spotted seatrout (i.e., May to September). Since spotted seatrout have a protracted spawning season, it is possible that males do not rely on the quality of their call to attract females because they have many opportunities to mate. On the other hand, red drum have a shortened spawning season, and therefore, males may rely heavily on the quality of their call to attract females because of limited mating opportunities.

We observed that sound production varied among tanks. More calling was detected in Tanks 1 and 2 as compared to Tank 3 (Table 1; Fig. 2). Tanks 1 and 2 contained more males than Tank 3, while Tank 3 contained more females (Table 1). Only male spotted seatrout have a sonic muscle and produce sound, which explains why more calling was detected in Tanks 1 and 2 as compared to Tank 3. In addition, spotted seatrout in Tanks 1 and 2 spawned more often and produced more eggs per gram of female biomass than seatrout in Tank 3, despite having close to twice the number of females in Tank 3 (Table 1; Fig. 5). These findings may indicate that having more males and males that are acoustically active in a spawning aggregation are key factors in enhancing reproductive output and sustaining populations.

CONCLUSIONS

This study is the first to record underwater sound and monitor spawning success of captive spotted seatrout populations over an entire simulated reproductive season. It reports quantitative data on the positive relationship between sound production and egg production. The fact that more calling and higher SPLs are associated with spawns that are more productive in captivity indicate that acoustic metrics can provide quantitative information on spawning in the wild. These data are important because it is not clear how accurately field sampling of eggs can be used to draw inferences about spawning activity because egg capture is likely to be affected by predator activity, water currents, and the efficiency of plankton tows. Hence, these findings are critical and provide instrumental information to scientists and managers who plan to use or are using passive acoustics as a tool to monitor spotted seatrout reproduction. Future studies can use this study as a framework to monitor the underwater soundscape continuously and long-term in order to understand the spatial and temporal patterns of spotted seatrout spawning and the possible impacts of anthropogenic stressors (e.g., climate change, noise pollution, and environmental pollutants) on these reproductive patterns.

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FIGURE LEGENDS

Figure. 1. Acoustic characterization of calls produced by wild caught spotted seatrout (*Cynoscion nebulosus*) held in captivity. Spotted seatrout produced three different call types. These calls were characterized as “drums”, “grunts”, or “staccatos” following similar nomenclature published in other studies (Mok & Gilmore, 1983; Sprague et al., 2000; Walters et al., 2009). (A) Spectrogram illustrating a staccato (labeled 1), a grunt (labeled 2), and a series of drums (labeled 3). Time domain of (B) a staccato, (C) a grunt, and (D) a drum call. Frequency domain of (E) a staccato, (F) a grunt, and (G) a drum call. Time and frequency domain figures correspond to the calls outlined in solid white lines in panel A. Brighter colors correspond to higher sound pressure levels.

Figure 2. Sound production by wild caught spotted seatrout (*Cynoscion nebulosus*) held in captivity throughout the entire study period. The number of drums, grunts, and staccatos in each 2 min ‘wav file’ was manually counted by an observer and plotted versus date with corresponding water temperatures for (A) Tank 1, (B) Tank 2, and (C) Tank 3. The numbers above the horizontal arrows indicate the number of hours of light present in the respective photoperiod. Boxes indicate rapid fluctuations in water temperature. Generally, abrupt rises in temperature were followed by an increase in calling, while abrupt drops were followed by a decrease in the amount of calling.

Figure 3. Daily patterns of sound production by spotted seatrout (*Cynoscion nebulosus*) in (A) Tank 1; (B) Tank 2; and (C) Tank 3. To examine these patterns, we determined the mean number of drums for each time interval (e.g., 12:00 to 12:02; 12:20 to 12:22; 12:40 to 12:42, 13:00 to

13:02, etc.) during the 14.5 h light photoperiod. The grey box indicates the time span of darkness during the 14.5 h light photoperiod. Standard deviations are reported as vertical bars.

Figure 4. Sound production and spawning of wild caught spotted seatrout (*Cynoscion nebulosus*) held in captivity throughout the entire study period. Calls per day and the number of eggs collected (i.e., the next morning) were plotted versus the date for (A) Tank 1, (B) Tank 2, and (C) Tank 3.

Figure 5. Tank comparisons of sound production and spawning for wild caught spotted seatrout (*Cynoscion nebulosus*) held in captivity. The total number of eggs collected per gram of female biomass versus (A) the total number of calls per days monitored and (B) the mean received sound pressure level (SPL) for Tank 1, Tank 2, and Tank 3. The total number of calls per days monitored was calculated by summing the total number of drums, grunts, and staccatos from 18:00 to 06:00 throughout the entire study period and then dividing this value by the number of days monitored. The mean SPL for each tank was determined by averaging all the 2 min SPLs from 18:00 to 06:00 over the entire study period.

Table 1(on next page)

Table 1. Tank summary of sound production and spawning events for captive spotted seatrout (*Cynoscion nebulosus*).

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Table 1. Tank summary of sound production and spawning events for captive spotted seatrout (*Cynoscion nebulosus*).

Tank Information	Tank 1	Tank 2	Tank 3	Means ± SE
No. of males	7	8	3	6 ± 2
Mean weight of males (g)	1,126 ± 320	1,314 ± 281	1,200 ± 194	1,213 ± 55
Mean length of males (mm)	469 ± 41	500 ± 36	484 ± 29	484 ± 9
No. of females	7	7	13	9 ± 2
Mean weight of females (g)	1,298 ± 194	1,953 ± 670	1,403 ± 209	1,551 ± 203
Mean length of females (mm)	497 ± 29	543 ± 70	502 ± 28	514 ± 15
Timeframe for data collection	4/13/12 - 12/19/12	4/13/12 - 11/21/12	4/13/12 - 11/21/12	NA
No. of days monitored	250	222	222	231 ± 9
Mean water temperature (°C)	26.4	24.9	26.0	25.8 ± 0.4
No. of spawns	81	13	3	32 ± 25
No. of spawns / days monitored	0.32	0.06	0.01	0.13 ± 0.10
Eggs collected	72,486,000	13,630,000	4,160,000	30,092,000 ± 21,372,558
Eggs collected / days monitored	289,944	61,396	18,739	123,360 ± 84,198
No. of drums	227,659	123,729	15,532	122,307 ± 61,240
No. of drums / days monitored	911	557	70	513 ± 244
Mean drums between 18:00 to 06:00 (no spawning)	708 ± 516	491 ± 467	59 ± 123	419 ± 191
Mean drums between 18:00 to 06:00 (spawning)	1,376 ± 538	1,624 ± 325	856 ± 442	1,285 ± 226
No. of grunts	13,109	6,105	1,786	7,000 ± 3,299
No. of grunts / days monitored	52	28	8	29 ± 13
Mean grunts between 18:00 to 06:00 (no spawning)	41 ± 32	25 ± 24	7 ± 15	24 ± 10
Mean grunts between 18:00 to 06:00 (spawning)	78 ± 45	69 ± 28	63 ± 51	70 ± 4
No. of staccatos	3,139	1,565	22	1,575 ± 900
No. of staccatos / days monitored	13	7	<1	10 ± 2
Mean staccatos between 18:00 to 06:00 (no spawning)	8 ± 8	6 ± 10	<1	7 ± 1
Mean staccatos between18:00 to 06:00 (spawning)	24 ± 14	29 ± 20	3 ± 4	19 ± 8
Mean SPL between 18:00 to 06:00 (no spawning)	120 ± 3	116 ± 3	113 ± 2	116 ± 2
Mean SPL between 18:00 to 06:00 (spawning)	124 ± 3	122 ± 2	118 ± 4	121 ± 2

NA = not applicable

SPL = received sound pressure level (dB re 1 uPa)

Means ± standard deviations reported for individual tanks.

Means ± standard errors of all four tanks.

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Table 2(on next page)

Table 2. Results of paired T-tests that tested if sound production and call structure differed significantly between non-spawning and spawning events for spotted seatrout (*Cynoscion nebulosus*).

Table 2. Results of paired T-tests that tested if sound production and call structure differed significantly between non-spawning and spawning events for spotted seatrout (*Cynoscion nebulosus*) held in captivity. P-values were statistically significant when P < 0.050.

Tank	Variable	t	d.f.	*P*
Tank 1	No. of drums	-9.406	243	<0.001
Tank 1	No. of grunts	-7.395	243	<0.001
Tank 1	No. of staccatos	-11.433	243	<0.001
Tank 1	Total No. of calls	-9.567	243	<0.001
Tank 1	Mean SPL	-8.996	243	<0.001
Tank 1	No. of pulses	-0.645	181	0.520
Tank 1	Call duration (s)	-1.460	181	0.146
Tank 2	No. of drums	-8.601	220	<0.001
Tank 2	No. of grunts	-6.307	220	<0.001
Tank 2	No. of staccatos	-7.480	220	<0.001
Tank 2	Total No. of calls	-8.641	220	<0.001
Tank 2	Mean SPL	-6.278	220	<0.001
Tank 2	No. of pulses	0.630	78	0.530
Tank 2	Call duration (s)	0.122	78	0.903
Tank 3	No. of drums	-10.600	220	<0.001
Tank 3	No. of grunts	-6.185	220	<0.001
Tank 3	No. of staccatos	-11.600	220	<0.001
Tank 3	Total No. of calls	-10.427	220	<0.001
Tank 3	Mean SPL	-4.206	220	<0.001
Tank 3	No. of pulses	-1.400	4	0.234
Tank 3	Call duration (s)	-0.863	4	0.437

SPL = received sound pressure level in dB re 1 μPa.

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Table 3(on next page)

Table 3. Results of linear regression that tested the significance of the amount of calling and sound pressure level in relation to spawning success of spotted seatrout (*Cynoscion nebulosus*).

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Table 3. Results of linear regression analysis that tested the significance of the amount of calling and sound pressure level in relation to spawning success of spotted seatrout (*Cynoscion nebulosus*) held in captivity. In all cases, the dependent variable is the number of eggs collected. *P*-values were statistically significant when $P < 0.050$.

Tank	Independent Variable	Fitted Equation	r^2	*P*	d.f.
Tank 1	Total no. of calls	$y = 402x - 104{,}522$	0.208	<0.001	243
Tank 1	Mean SPL	$y = 74{,}752x - 8{,}745{,}163$	0.245	<0.001	243
Tank 2	Total no. of calls	$y = 235x - 77{,}917$	0.065	<0.001	220
Tank 2	Mean SPL	$y = 36{,}452x - 4{,}193{,}465$	0.046	0.001	220
Tank 3	Total no. of calls	$y = 0.074x + 41{,}116.946$	0.040	0.003	220
Tank 3	Mean SPL	$y = 31{,}294x - 3{,}533{,}082$	0.112	<0.001	220

SPL = received sound pressure level in dB re 1 μPa.

1

Figure 1(on next page)

Figure. 1. Acoustic characterization of calls produced by wild caught spotted seatrout (*Cynoscion nebulosus*) held in captivity.

Figure. 1. Acoustic characterization of calls produced by wild caught spotted seatrout (*Cynoscion nebulosus*) held in captivity. Spotted seatrout produced three different call types. These calls were characterized as “drums”, “grunts”, or “staccatos” following similar nomenclature published in other studies (Mok & Gilmore, 1983; Sprague et al., 2000; Walters et al., 2009). (A) Spectrogram illustrating a staccato (labeled 1), a grunt (labeled 2), and a series of drums (labeled 3). Time domain of (B) a staccato, (C) a grunt, and (D) a drum call. Frequency domain of (E) a staccato, (F) a grunt, and (G) a drum call. Time and frequency domain figures correspond to the calls outlined in solid white lines in panel A. Brighter colors correspond to higher sound pressure levels.

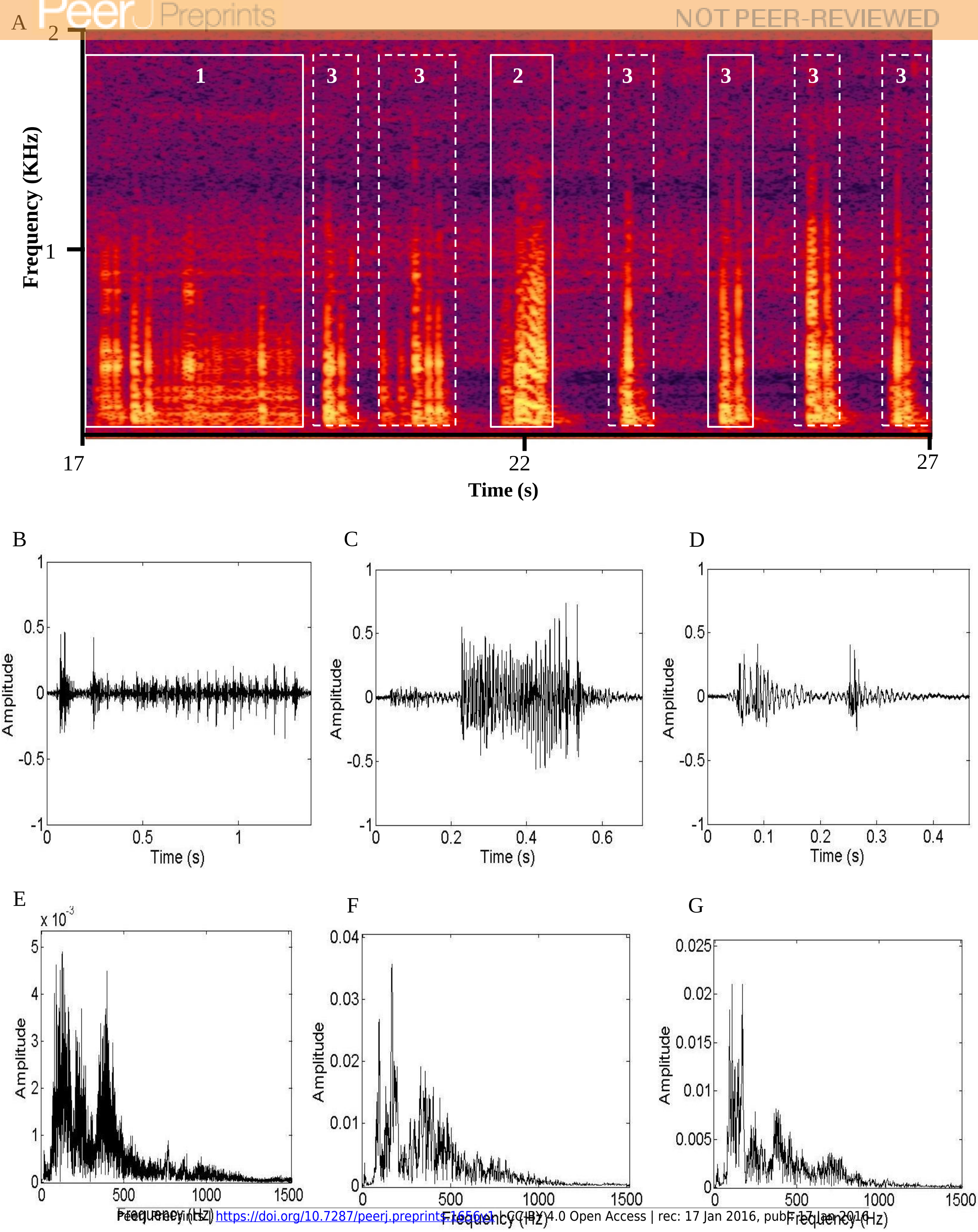

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Figure 2(on next page)

Figure 2. Sound production by wild caught spotted seatrout (*Cynoscion nebulosus*) held in captivity throughout the entire study period.

Figure 2. Sound production by wild caught spotted seatrout (*Cynoscion nebulosus*) held in captivity throughout the entire study period. The number of drums, grunts, and staccatos in each 2 min 'wav file' was manually counted by an observer and plotted versus date with corresponding water temperatures for (A) Tank 1, (B) Tank 2, and (C) Tank 3. The numbers above the horizontal arrows indicate the number of hours of light present in the respective photoperiod. Boxes indicate rapid fluctuations in water temperature. Generally, abrupt rises in temperature were followed by an increase in calling, while abrupt drops were followed by a decrease in the amount of calling.

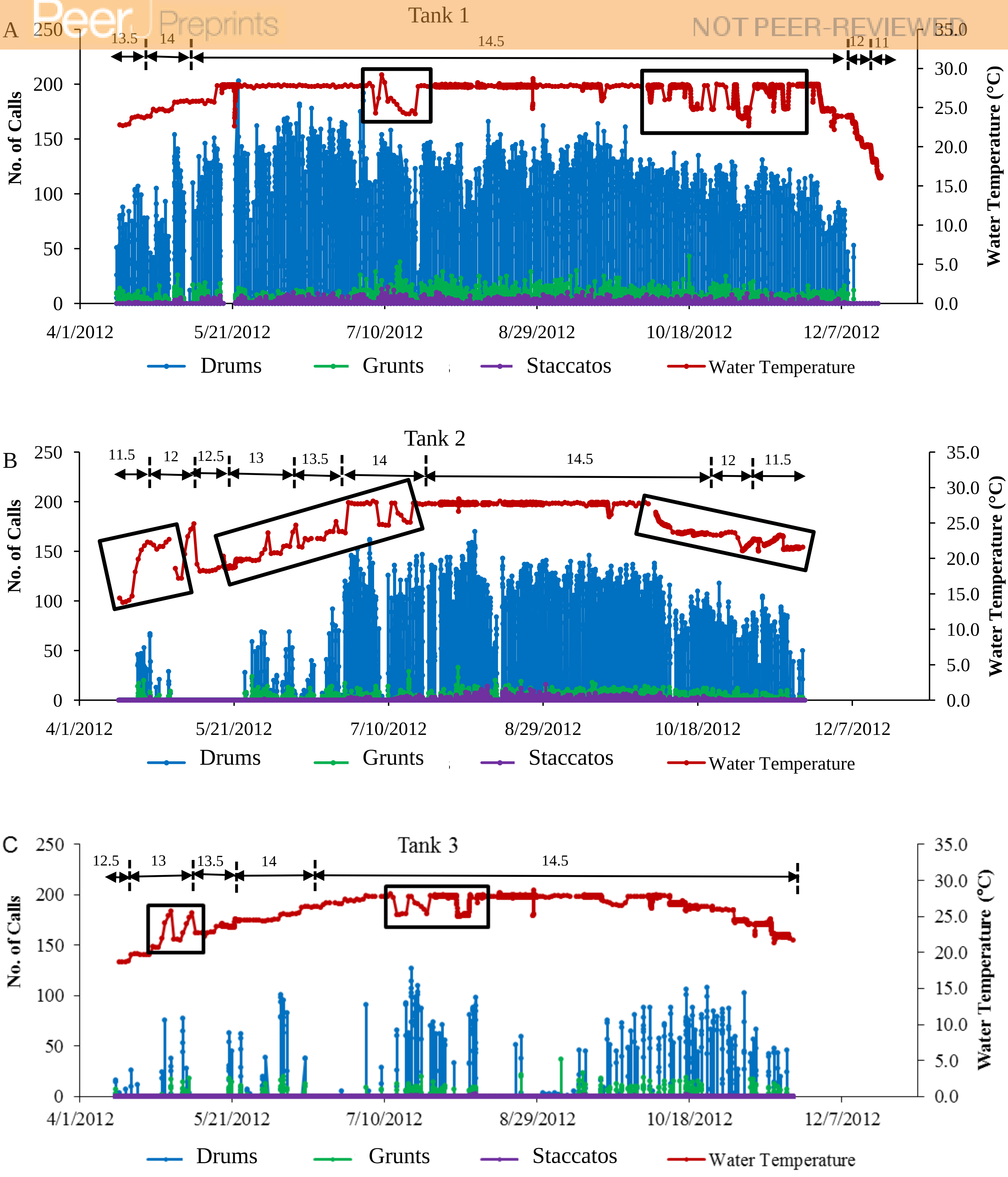

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Figure 3(on next page)

Figure 3. Daily patterns of sound production by spotted seatrout (*Cynoscion nebulosus)*.

Figure 3. Daily patterns of sound production by spotted seatrout (*Cynoscion nebulosus*) in (A) Tank 1; (B) Tank 2; and (C) Tank 3. To examine these patterns, we determined the mean number of drums for each time interval (e.g., 12:00 to 12:02; 12:20 to 12:22; 12:40 to 12:42, 13:00 to 13:02, etc.) during the 14.5 h light photoperiod. The grey box indicates the time span of darkness during the 14.5 h light photoperiod. Standard deviations are reported as vertical bars.

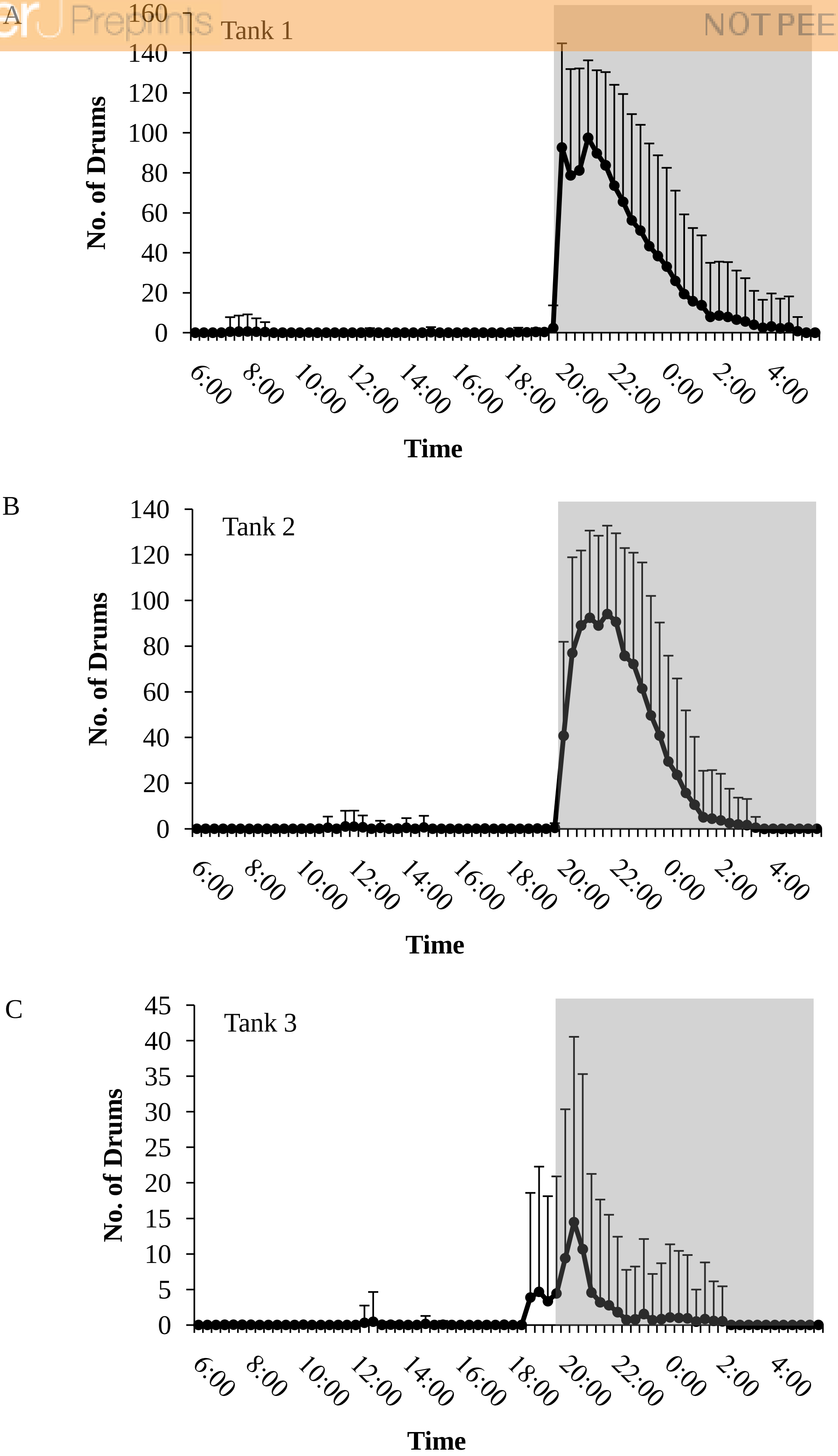
A
Tank 1
No. of Drums
160
140
120
100
80
60
40
20
0
6:00
8:00
10:00
12:00
14:00
16:00
18:00
20:00
22:00
0:00
2:00
4:00
Time
B
Tank 2
No. of Drums
140
120
100
80
60
40
20
0
6:00
8:00
10:00
12:00
14:00
16:00
18:00
20:00
22:00
0:00
2:00
4:00
Time
C
Tank 3
No. of Drums
45
40
35
30
25
20
15
10
5
0
6:00
8:00
10:00
12:00
14:00
16:00
18:00
20:00
22:00
0:00
2:00
4:00
Time

Figure 4(on next page)

Figure 4. Sound production and spawning of wild caught spotted seatrout (*Cynoscion nebulosus*) held in captivity throughout the entire study period.

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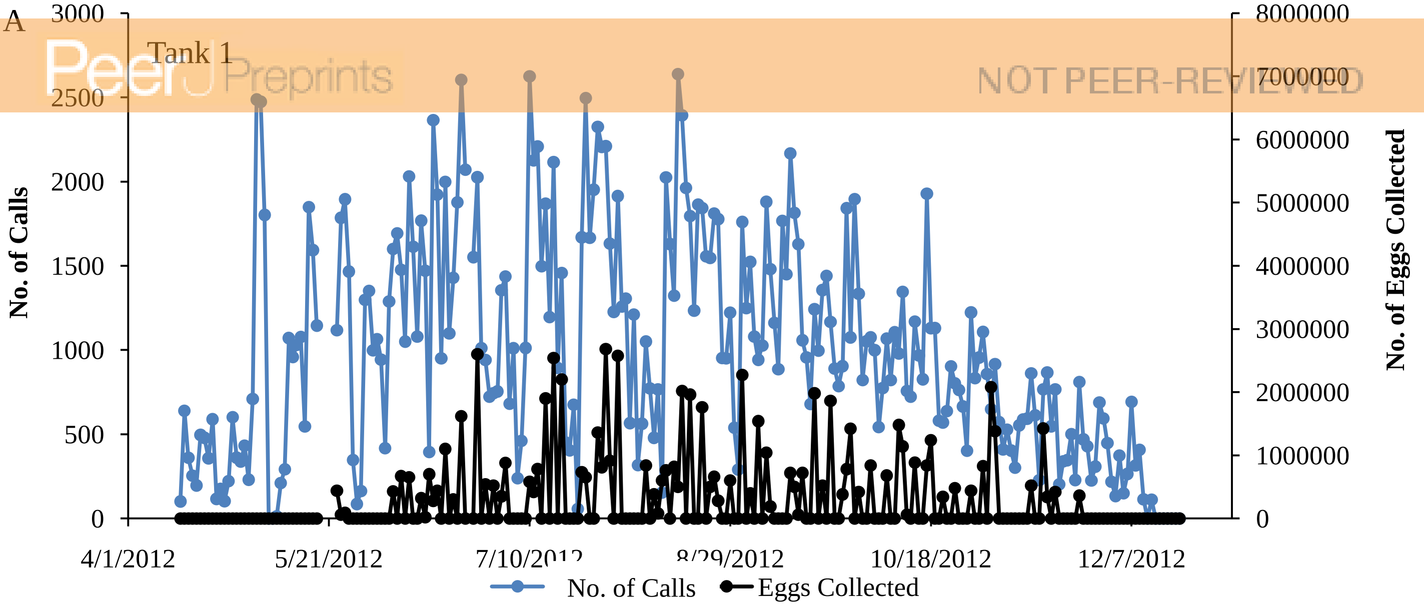
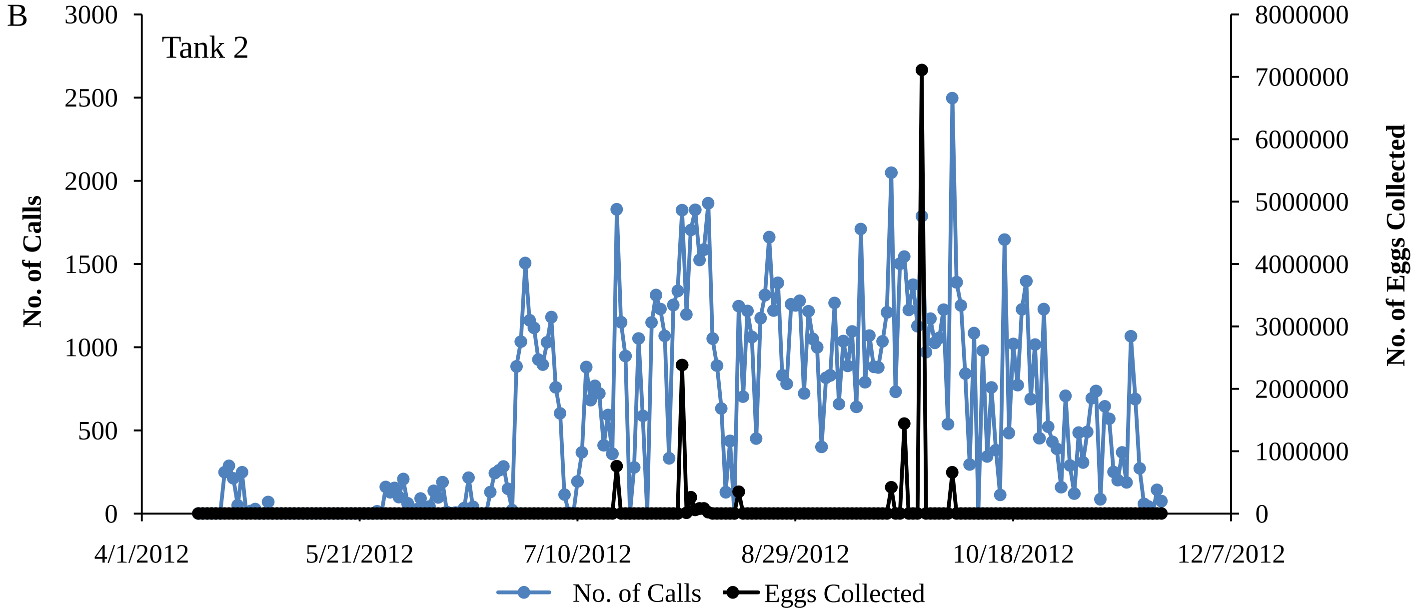

A
Tank 1
No. of Calls
No. of Eggs Collected
0
500
1000
1500
2000
2500
3000
0
1000000
2000000
3000000
4000000
5000000
6000000
7000000
8000000
4/1/2012
5/21/2012
7/10/2012
8/29/2012
10/18/2012
12/7/2012
No. of Calls
Eggs Collected


B
Tank 2
No. of Calls
No. of Eggs Collected
0
500
1000
1500
2000
2500
3000
0
1000000
2000000
3000000
4000000
5000000
6000000
7000000
8000000
4/1/2012
5/21/2012
7/10/2012
8/29/2012
10/18/2012
12/7/2012
No. of Calls
Eggs Collected

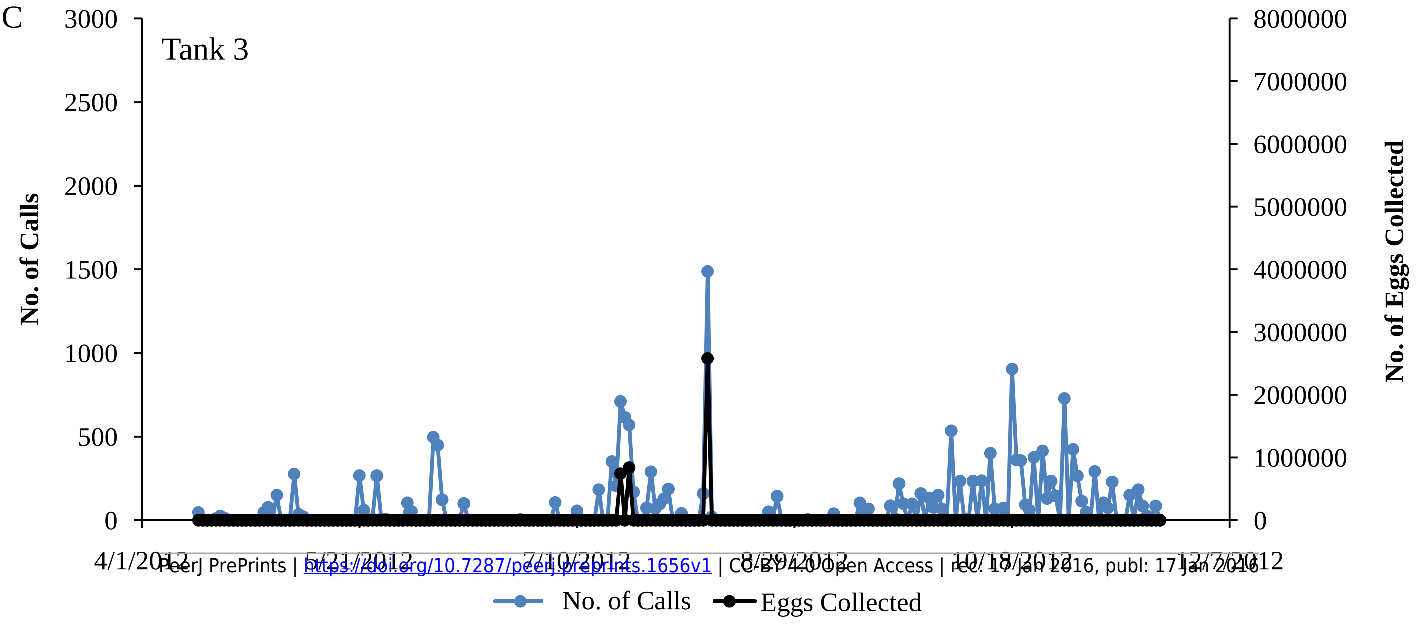

C
Tank 3
No. of Calls
No. of Eggs Collected
0
500
1000
1500
2000
2500
3000
0
1000000
2000000
3000000
4000000
5000000
6000000
7000000
8000000
4/1/2012
5/21/2012
7/10/2012
8/29/2012
10/18/2012
12/7/2012
No. of Calls
Eggs Collected

Figure 5(on next page)

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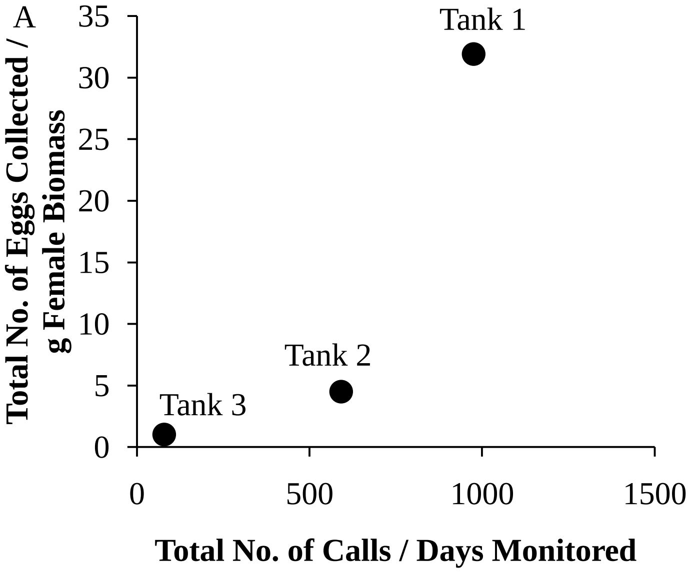

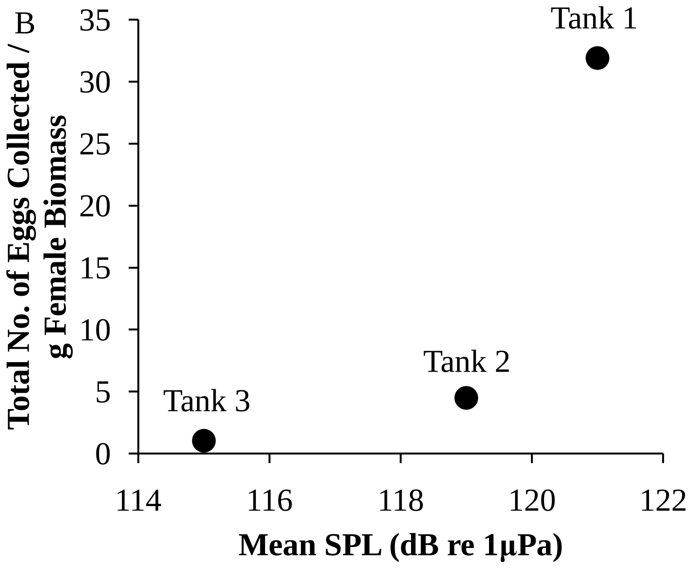

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