

# Production of live offspring following unilateral (left) ovariectomized *Potamotrygon* rays (*Potamotrygon castexi*, *Potamotrygon leopoldi*, and *Potamotrygon motoro*)

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Received January 26, 2023

Accepted March 10, 2023

doi.org/10.2460/javma.23.01.0031

## OBJECTIVE

To evaluate the surgical technique and subsequent clinical observations (reproductive and ultrasound findings) of left unilateral ovariectomy in 3 species of *Potamotrygon* rays—*Potamotrygon castexi*, *Potamotrygon leopoldi*, and *Potamotrygon motoro*—for reproductive management.

## ANIMALS

Between 2018 and 2019, multiple *Potamotrygon* rays (*P castexi*, n = 1; *P leopoldi*, 1; *P motoro*, 6) underwent left ovariectomies to evaluate this technique for reproductive management.

## PROCEDURES

At time of surgery, patient age ranged from juvenile to adult. Rays were anesthetized with MS222 buffered with sodium bicarbonate, and a left craniodorsal surgical approach was made to isolate and excise the left ovary. All rays had uneventful recoveries. Eight unilateral ovariectomized females and 6 males were combined in a mixed-species freshwater touch pool of *Potamotrygon* rays and teleost species.

## RESULTS

In December 2020, 3 live and 1 premature autolyzed pup were noted in the habitat. The following day, the adult females were examined via ultrasound and separated from the males. Four dams were identified that produced 8 viable offspring and 4 premature abortions. A large right ovary was observed in all females, with no evidence of left ovarian tissue present via ultrasound.

## CLINICAL RELEVANCE

Previous histologic evaluation of freshwater ray ovarian tissue suggests both ovaries may be functionally active yet maintain left dominance like some other elasmobranch species. This manuscript provides proof the right ovary alone can produce live offspring. Furthermore, the enlarged right ovary observed in these females suggests that removal of the left ovary may result in compensatory enlargement of the right ovary.

A large display aquarium acquired 3 species of *Potamotrygon* rays between 2015 and 2018 from customs confiscations by US Fish and Wildlife. Fourteen of these *Potamotrygon* rays were housed in a mixed-species 23,000-L indoor freshwater touch pool. The exhibit was mix gendered, and contraception was required to prevent reproduction due to inability to deaccession any potential offspring. The general recommendation for contraception in this species is gender separation, with no other effective documented methods (sur-

gical or hormonal).<sup>1</sup> Due to space constraints of the institution, gender separation long-term was not a viable option. Administration of gonadotropin-releasing hormone (GnRH) analogs such as Suprelorin to contracept elasmobranchs has had poor to limited success,<sup>2,3,4</sup> presumptively due to the 7 different types of GnRH found in elasmobranch species.<sup>5,6,7</sup> In *Potamotrygon* sp, 4.7 mg and 9.4 mg Suprelorin implants (GnRH analog-protein conjugate) failed to reduce testosterone levels, sperm quality, or sperm quantity.<sup>8</sup> Left ovariectomies have been success-

ful for contraceptive management of other batoid species, including southern stingrays (*Dasyatis americana*).<sup>9</sup> This report outlines the surgical technique and subsequent clinical observations (reproductive and ultrasound findings) following left unilateral ovariectomy in 3 species of *Potamotrygon* rays, *Potamotrygon castexi*, *Potamotrygon leopoldi*, and *Potamotrygon motoro*, which will aid future clinicians in reproductive management of these species.

## Materials and Methods

Prior to surgery, females were kept separate from males in a series of variably sized off-exhibit or quarantine holding systems at a large display aquaria and had no major medical history. Ray diets consisted of smelt, capelin, and trout feed twice daily, targeting 2% to 3% body weight per week. Surgical procedures were pursued on the basis of clinical need; therefore, IACUC or other approval was not necessary. Left ovariectomies were performed between 2018 and 2019 on all 8 female *Potamotrygon* rays in the collection ranging in age from juvenile to adult (*P castexi*, n = 1; *P leopoldi*, 1; *P motoro*, 6) in efforts to provide contraception. At the time of surgery, rays weighed between 0.5 and 9.55 kg, had disc widths ranging from 36 to 60 cm, and were presumed healthy by physical exam. Presurgical ultrasound exams of all females included in the study noted normal-sized left ovaries and small or nonvisible right ovaries via experienced ultrasonographer. Rays were anesthetized with 85 ppm MS222 buffered with 170 ppm sodium bicarbonate. After a 6-minute induction, rays were sufficiently anesthetized, as determined by lack of response to stimulus while maintaining spontaneous gilling. They were moved to a surgical wet table with recirculating bifurcated ventilation placed in the mouth, resulting in flow over the gills for anesthesia maintenance, and positioned in ventral recumbency. Aseptic preparation was performed with dilute betadine or saline rinse over the planned surgical site. The surgery site was draped, and the drape was sutured with a single interrupted suture of 3-0 polydioxanone suture (PDS) at the cranial and caudal aspect to prevent movement during surgery. The surgical technique followed that reported for southern

rays.<sup>9</sup> A left craniodorsal surgical approach was made to isolate and excise the left ovary. An approximately 4 cm skin incision was made parallel to the spinal column and approximately 1 cm lateral to the dorsal lumbar muscles. The underlying musculature and peritoneum were incised with a No. 15 blade and extended the length of the skin incision. The cranial aspect of the ovary was located and clamped with a curved hemostat. Two Miller knots using 3-0 PDS (MWI Veterinary Supply Inc) were placed at the ovarian mesentery to ligate the blood supply to the ovary. The suspensory ligament was bluntly dissected, and the ovary and epigonal tissue were removed, as they could not be readily distinguished or separated. The peritoneum was closed using simple interrupted sutures, and the skin was closed with horizontal mattress sutures, using 3-0 PDS for both layers.

Supplemental care included intraoperative IM injections of 20 mg of florfenicol/kg and 2 mg of ketoprofen/kg. All rays tolerated the approximately 1-hour procedure and had uneventful recoveries. After an approximately 4-week postoperative period, skin sutures were removed and females were cleared by staff veterinarian for cohabitation with males.

## Results

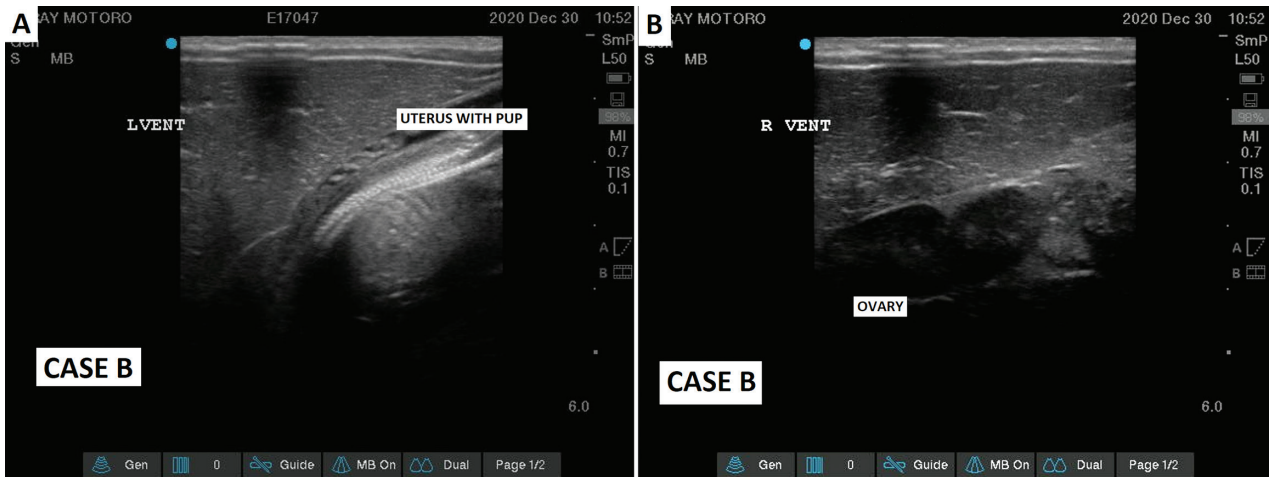
Ovariectomized females from this study and 6 males were combined in a mixed-species freshwater touch pool of *Potamotrygon* rays and teleost species in July 2020. On December 21, 2020, 3 live and 1 premature autolyzed pup were found in the touch pool habitat during husbandry checks. The following day, the females were isolated from the males. At that time, all females were imaged via transcoelomic ultrasound to assess pregnancy status. Four of 8 were identified to be currently or recently gravid and produced 8 viable offspring. One of the 4 dams required hysteroscopy and manual removal of 4 immature nonviable pups. Surgery dates and subsequent pregnancies and offspring are outlined (**Table 1**). The consistent notable finding of all left ovariectomized females on ultrasound examination was a large right ovary with no evidence of left ovarian tissue (**Figure 1**). Of note, 2 rays with consistent right ovarian enlargement did not have uterine findings suggestive of recent pregnancy.

**Table 1**—Acquisition and surgery dates and subsequent pregnancies and offspring of the rays of the present report observed in December 2020.

Case ID	Species	Acquisition	Surgery date	Pregnant 2020 (Y/N)	Viable offspring (Y/N)
A	<i>Potamotrygon castexi</i>	2017	27-Feb-19	N	N
B	<i>Potamotrygon leopoldi</i>	2017	2-Nov-18	Y	Y
C	<i>Potamotrygon motoro</i>	2017	2-Nov-18	Y	Y
D	<i>P motoro</i>	2018	2-Nov-18	Y	N
E	<i>P motoro</i>	2017	2-Nov-18	N	N
F	<i>P motoro</i>	2015	23-May-19	U	U
G	<i>P motoro</i>	2015	17-Dec-19	Y <sup>1</sup>	Y <sup>1</sup>
H	<i>P motoro</i>	2015	27-Feb-19	N	N

N = No. U = Unknown if offspring. Y = Yes.

<sup>1</sup>Presumed first dam of found offspring due to presence of trophonemata in absence of fetus on ultrasound.



**Figure 1**—Ultrasound images collected from a *Potamotrygon* ray scanned from ventrum under manual restraint. A—Ultrasound image showing uterus and pup wing. B—Ultrasound image showing right ovary deep to the liver.

## Discussion

Historically, *Potamotrygon* sp were believed to be left ovary dominant with vestigial right ovaries.<sup>10</sup> Histologic analysis of the reproductive tract from various species of *Potamotrygon* rays, including *P motoro*, revealed asymmetric ovaries (left notably larger than right), where both ovaries contained germinal epithelium, ovarian stroma, oogonia, and follicles in different stages of development. However, the right ovary was predominantly composed of germinal epithelium and some atretic follicles while the left ovary was composed of corpora lutea and some atretic follicles, thereby suggesting ovulation occurred only on the left side.<sup>11</sup> This study noted that although the right ovary was smaller in size with limited follicular development, it might be functional in potamotrygonids.<sup>11</sup> A similar evaluation on *Potamotrygon magdalenae* reported similar findings whereby the reproductive tract was bilaterally functional but suspected the left ovary predominantly contributed to reproduction, as it had an increased number of oocytes and embryos in comparison to the right ovary.<sup>12</sup> The findings of this study corroborated the speculation of Da Silva et al<sup>3</sup> that the right ovary is reproductively active in *Potamotrygon* rays, particularly in *P castexi*, *P leopoldi*, and *P motoro*, identifying several cases of successful reproduction by a right ovary in the absence of a left.

In the rays of this report, a 1- to 2-year time period elapsed between left ovariectomy and observation of offspring or confirmation of late-term pregnancy. However, males and females only cohabitated 6 months prior to observation of offspring. Gestation time varies by species of *Potamotrygon*, ranging from 3 to 11 months while having 1 to 21 pups/litter. In human care, gestation times of 3 months in *P motoro* and 4 months in *Potamotrygon tigrina* and *P leopoldi* have been observed.<sup>1</sup> This species is not known to be parthenogenic. The enlarged right ovary seen in these females following left ovariectomy suggests that removal of the left ovary may

result in compensatory enlargement of the right ovary and maintenance of fecundity in this species. Given the timeline of surgery and delayed reintroduction to males, it is unclear whether the access to males specifically drove the enlargement of the remaining right ovary. It is equally possible an innate biological process was already at work, as the right ovary is reported to regularly undergo folliculogenesis in this species with the left ovary present.<sup>11</sup>

Two *P motoro* (cases F and G; Table 1) had consistent reproductive findings on ultrasound (enlarged right ovaries); however, offspring were not confirmed/observed. As there were numerous specimens cohabitating during this time, it is possible live or aborted offspring were consumed by a male ray or teleost in the habitat. Male potamotrygonids have been reported to traumatize and/or kill pups.<sup>1</sup> Should one be trying to breed *Potamotrygon* rays, it is recommended to separate the gravid individual from conspecifics in advance of parturition.

Unilateral ovariectomy was not a reasonable method of contraception in *Potamotrygon* species in this study, and a bilateral approach should be considered if permanent contraception is desired in potamotrygonid rays. Authors caution that a bilateral ovariectomy may remove the entire epigonal organ if the surgeon cannot discern the ovary from epigonal; however, partial epigonadectomies have been successful in other species. The observation of a reproductively functional right ovary warrants further investigations in other elasmobranch species with ovarian asymmetry, as unilateral surgical contraception may not be a reliable option in those elasmobranch species. Alternative contraception methods utilized in elasmobranch species include deslorelin (hormonal implant) contraception or maintaining separate sexed habitats. Further studies are necessary to fully evaluate efficacy of GnRH implants in females for contraception or males for mitigating male-female breeding aggression and male-male aggression. Thus far, trials with hormone management in elasmobranchs have provided mediocre results,

and additional research is warranted. Male *Potamotrygon* sp have not been successfully contracepted with hormonal implants thus far.<sup>8</sup> Separate sexed habitats still prove to be the most reliable method of reproductive management in *Potamotrygon* species.

## Acknowledgments

No external funding was used in this study. The authors declare that there were no conflicts of interest.

The authors thank the Georgia Aquarium veterinary technicians for their assistance in the medical care of these individuals as well as the fish and invertebrate animal care staff for their daily care. The authors thank Dr. Linda Penfold for support and subject matter expertise. Additional appreciation to Dr. Robert George for his guidance in performing successful left ovariectomies in these species.

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