Acta Scientiarum. Biological Sciences
ISSN: 1679-9283
eduem@uem.br
Universidade Estadual de Maringá
Brasil

Yasui, Anne Marie; Shigueki Yasui, George; Shimoda, Eduardo; Pinto Ribeiro-Filho, Oswaldo
Concentration of anesthetic for long-term exposure and the effects of inter-suture distance in the goldfish Carassius auratus
Acta Scientiarum. Biological Sciences, vol. 31, núm. 2, 2009, pp. 203-207
Universidade Estadual de Maringá
.png, Brasil

DOI: 10.4025/actascibiolsci.v31i2.802

Concentration of anesthetic for long-term exposure and the effects of inter-suture distance in the goldfish *Carassius auratus*

Anne Marie Yasui[1*], George Shigueki Yasui[2], Eduardo Shimoda[3] and Oswaldo Pinto Ribeiro-Filho[4]

*[1]Clínica VetCenter, Rua Ari Parreira, 209, 23820-300, Centro, Itaguaí, Rio de Janeiro, Brazil. [2]Laboratory of Aquaculture Genetics and Genomics, Hokkaido University, Hakodate, Japan. [3]Instituto Candido Mendes, Universidade Cândido Mendes, Campos do Goytacazes, Rio de Janeiro, Brazil. [4]Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. *Author for correspondence. E-mail: anneyasui@terra.com.br*

ABSTRACT. This study aimed to determine the minimum concentration of 2-phenoxyethanol for long-term exposure and evaluate the effect of inter-suture distance on wound healing in the goldfish *Carassius auratus*. Twenty adult goldfish (standard length = 12.4 ± 1.1 cm; weight = 58.7 ± 17.2 g) were anesthetized in 2-phenoxiethanol at 1.2‰ and placed in an anesthesia delivery system at the following concentrations of 2-phenoxiethanol: 0.0 (control); 0.1; 0.2; 0.3 and 0.4‰, and the duration of sedation was measured. Fifteen days later, fishes were anesthetized using the same procedure, and a 36 mm incision was performed in the ventro-lateral region. The incision was sutured using a simple-interrupted pattern with 3, 6 or 9 mm as inter-suture distances. Results demonstrated that 2-phenoxiethanol at 0.4‰ maintain the sedation for surgical procedures up to 60 minutes, and 9 mm as inter-suture distance optimized the wound healing in goldfish.

Key words: anesthetic, surgery, healing, fish, suture.

RESUMO. Concentração de anestésico para exposição em longo prazo e efeitos da distância entre suturas em kinguyo *Carassius auratus*. Objetivou-se com o presente trabalho obter a concentração mínima de 2-phenoxyethanol para a exposição em longo prazo e avaliar o efeito da distância entre suturas (DES) no tempo de cicatrização de incisão em kinguio *Carassius auratus*. Vinte kinguyos adultos (comprimento-padrão = 12,39 ± 1,1 cm; peso = 58,71 ± 17,24 g) foram anestesiados em 2-phenoxiethanol a 1,2‰ e então colocados em um sistema de recirculação de anestesia contendo 2-phenoxiethanol nas seguintes concentrações: 0,0 (controle); 0,1; 0,2; 0,3 e 0,4‰, e foi observada a duração da sedação. Quinze dias depois, os peixes foram novamente anestesiados, utilizando-se o mesmo procedimento, e então foi realizada uma incisão de 36 mm na região ventro-lateral. A incisão foi suturada utilizando-se o padrão interrompido-simples, em nylon, com distância entre suturas de 3, 6 e 9 mm. Os resultados demonstram que 0,4‰ 2-phenoxiethanol permite a manutenção do estado de sedação para procedimentos cirúrgicos acima de 60 min., e 9 mm como distância entre suturas otimiza a cicatrização em kinguyo.

Palavras-chave: anestésico, cirurgia, cicatrização, peixe, sutura.

Introduction

Surgical techniques in fish permits several applications as the implantation of teletransmitters and fistulas (PAUKERT et al., 2001; COOKE et al., 2005), castration (JENKINS; DODD, 1982; BART; DUNHAN, 1990; KOBAYASHI et al., 2000), organ transplantation (NAGLER et al., 2001; CLOUD, 2003), biopsy (WOOSTER et al., 1993; HILSDORF et al., 1999; PETERSON et al., 2005) as well as simple procedures such as the removal of external parasites and individual tagging (BERRY JR. et al., 1991; BANDILLA et al., 2005; TEIXEIRA; CORTEZ, 2007). However, fish species are numerous and the anatomic-physiologic aspects are species-specific. It results in a lack of basic information on literature for surgical interventions in most fish species.

Sedation is one of the main aspects to consider during surgical procedures. Furthermore, it is required by several legal restrictions that aim to reduce to a minimum any pain, suffering or stress imposed to the animals (SCHNAIDER; SOUZA, 2003). In fish, sedation can be induced basically using three methodologies: 1) by intravenous vehiculation of the anesthetic; 2) by hypothermy; and 3) by immersion of fish in anesthetic solutions. For convenience, the last mentioned procedure has been the most employed.

Surgeries of short duration (< 5 min.) are realized with the animals immersed into the water. After finishing the surgery fish are placed in well aerated water for recovering. On contrary, in interventions that require longer periods, it is recommended the utilization of long-term delivery of anesthetic by forced ventilation (SMITH; BELL, 1967; STOSKOPF, 1993; HARMS; LEWBART, 2000). This procedure permits the performance of surgery in terrestrial environment. In this methodology, sedation is divided into two stages: the induction, in which the fish is immersed in a solution of anesthetic at higher dosages; and maintenance, at lower dosages administrated through the gills, to prolong the effects of the previous dosage. Maintenance dosages lower than the adequate range will permit the recovering of the fish during surgery. On the other hand, higher concentrations may induce death.

In surgery, wound suturing also requires attention since it is related with healing. Suturing must provide an effective healing and reduce the post-operatory period.

Besides the supra mentioned aspects, we aimed with the present study to obtain the optimum dosage of 2-phenoxyethanol for maintenance of sedation and evaluate the effects of inter-suture distance on wound healing in goldfish *Carassius auratus*.

Material and methods

The goldfish (*Carassius auratus*) employed in our experiments were obtained from a local fish dealer. These experiments were conducted at Federal University of Viçosa - Department of Animal Biology, Minas Gerais State, Brazil, from May to July 2007.

Experiment 1: Evaluation of maintenance dosage for 2-phenoxiethanol

Twenty adult goldfish (12.39 ± 1.1 mm; 58.71 ± 17.2 g) were anesthetized in 2-phenoxiethanol at 1.2‰ until achieving deep anesthesia (no movement, incapability to return to upright position, no responsive to tactile stimuli, no opercular ventilation). After this period, fishes were placed in an anesthesia delivery system (HARMS; LEWBART, 2000). The anesthetic was provided by forced ventilation using a silicone tube with 5.7 mm diameter at a flow rate of 240 L h^{-1}. We evaluated the following concentrations of anesthetic: 0.0‰ (control); 0.1‰; 0.2‰; 0.3‰ and 0.4‰ 2-phenoxyethanol dissolved in dechlorinated tap water. Four fish were utilized for each concentration. Both room and water temperatures were maintained around 21ºC.

After anesthetization, stimuli (compression) along the ventral region were performed periodically in order to evaluate the efficacy of sedation. When fish regained movement, the duration of sedation was measured and fish were placed in a 200 L aquaria containing well aerated water with methylene blue at 0.01% for recovering.

Fish were maintained under sedation at a maximum period of 60 minutes.

Experiment 2: Effects of inter-suture distance on healing time

Fifteen days after concluding experiment 1, twelve goldfish (12.1 ± 1.3 cm; 63.3 ± 18.3 g) were starved for 48h and sedated using our best results observed previously (experiment 1). Three lines of scales were removed from the ventro-lateral region using forceps. A 36 mm incision was performed using scalpel and sutured with a simple-interrupted pattern using a 4-0 nylon monofilament (Ethicon Inc.). The inter-suture distances (ISD) employed were 3, 6 and 9 mm, what resulted in 3 treatments with four replications. During the surgical procedures the skin was kept moist to prevent dehydration.

The duration of surgical procedures (removal of scales, incision and suturing) was measured. The wound was disinfected with a solution of methylene-blue (1%) and the fishes were placed in a 200 L aquaria (6 fish $tank^{-1}$) containing well aerated water with NaCl (0.75%) at 21ºC. During the first two days, fishes were maintained in dark conditions and no food was provided. From the third day after surgery, we increased gradually the photoperiod to 14h Light:10h Dark and feeding rate from 0.5% to 3% of tank biomass (commercial carp pellet 36% crude protein).

At four-days intervals, all fishes were anesthetized (1.2‰ 2-phenoxyethanol) and the incision was observed and photographed. The wound opening, the level of wound inflammation and suture integrity was evaluated visually (Table 1), similarly as described by Wagner et al. (2000). After observation, the wound was disinfected using methylene blue 1% and fish were returned to the tanks.

Table 1. Scale for scoring the level of suture integrity, wound inflammation and wound healing based on visual inspection.

Score	Criteria for scoring		
	Suture integrity	Wound inflammation	Wound healing
0	All sutures are lost or in the skin region.	No inflammation.	Completely closed wound.
1	< 25% of sutures are lost or in the skin region.	Little inflammation along the incision site (< 25%).	< 25% of wound open.
2	< 50% of sutures are lost or in the skin region.	Moderate inflammation (< 50% of incision inflamed).	< 50% of wound open.
3	< 75% of sutures are lost or in the skin region.	Moderate to high inflammation (< 75% of incision inflamed).	< 75% of wound open.
4	< 100% of sutures are lost or in the skin region.	High inflammation (< 100% of incision inflamed).	< 100% of wound open.
5	100% of sutures are in good conditions	Severe inflammation (> 100% of incision inflamed).	Completely open wound.

Observation was interrupted after 20 days from suturing, when all sutures were then removed.

Statistics

Results are shown as mean ± SD. Average data obtained in experiments 1 and 2 were compared by means of Anova succeeded by a Tukey multiple range test. Pearson's correlation was utilized to evaluate dosages of anesthesia and biometric parameters. In all cases it was considered $p < 0.05$.

Results and discussion

The time period for reaching sedation at 1.2‰ 2-phenoxyethanol was 2:57 ± 0:32 min. This parameter was correlated with standard-length (r = 0.33) and body weight (r = 0.39). These data are in agreement with Mohamed (1999) and Inoue et al. (2004) who compared several concentrations of 2-phenoxyethanol in other teleosts and observed that at 0.6-0.7‰ the fishes reached sedation in approximately one minute. Mylonas et al. (2005) used the same anesthetic at concentrations ranging from 4 to 6 mg L^{-1} for the sea bass and sea bream, with an induction time shorter than 3 min.

The duration of sedation at maintenance dosages in control group were 1:46 ± 0:23 min., increasing to 4:24 ± 1:15 min. at 0.1‰ and maintained at the same level at 0.2‰ (3:43 ± 1:15 min.). At 0.3‰, the duration of sedation increased to 7:41 ± 0:52 min., and when we used the highest concentration (0.4‰) the duration increased abruptly to 60 minutes (Figure 1).

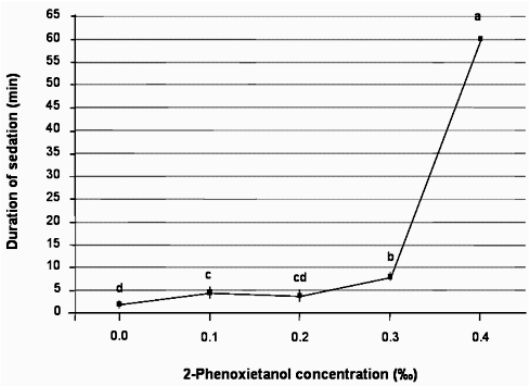

Figure 1. Effects of 2-phenoxyethanol concentration on sedation of goldfish *Carassius auratus*. Fishes were anesthetized at 1.2‰ and placed in an anesthesia delivery system at 0.1, 0.2, 0.3 and 0.4‰ of 2-phenoxyethanol and the duration of sedation was measured. Identical letters above the values denotes no statistical differences by the Tukey test ($p < 0.05$).

Harms and Lewbart (2000) suggests a general formulation for sedation in fish. For tricaine-methane-sulphonate (MS-222) they recommended an induction dosage at 100-200 mg L^{-1} and 50-100 mg L^{-1} for maintenance. In the case of eugenol these authors suggested a concentration ranging from 100 to 40 mg L^{-1} respectively for induction and maintenance. However, these concentrations have a variation according to fish specie, size or age and environmental conditions (ROSS; GEDDES, 1979; HARMS; LEWBART, 2000). Mylonas et al. (2005) compared the effects of 2-phenoxyethanol in the sea bream and sea bass, and observed a correlation between the species and water temperature during sedation. Bart and Dunham (1990) observed better survival rates in channel catfish anesthetized at 12ºC (61%) when compared to 23ºC (53%), similarly as suggested by Smith and Bell (1967) who have reported that MS-222 was more effective at lower temperatures. In the case of our study, all experiments were conducted at room temperature set at 21ºC and both, room and water temperatures were maintained under isotherm conditions.

Although we could increase the exposure time longer than 60 min. at 0.4‰ 2-phenoxiethanol, we interrupted the treatment since this period is sufficient for most of surgical interventions. In addition, longer exposure could threat the animal welfare. In all cases, suturing was followed by disinfecting and the fish completely recovered the equilibrium in a period shorter than 10 minutes.

As expected, the duration of surgical procedures had an inverse relation with inter-suture distance. The duration of surgical procedures involved the removal of scales, performing of incision and suturing. At 3 mm as inter-suture distance the duration was 21:48 ± 0:32 minutes, decreasing to 11:03 ± 1:57 minutes at 6 mm and the shortest duration was observed at 9 mm of inter-suture distance (06:34 ± 0:33 minutes). In all treatments the duration of surgical procedures were statistically different.

Data regarding wound healing, inflammation and suture integrity are shown on Table 2. The wounds healed completely in all treatments at 16th day after surgery, and 9 mm as inter-suture distance provided the fastest healing. Although the utilization of 9 mm as inter-suture distances optimized the healing time in the goldfish, other investigations should be conducted in order to verify this parameter in other species, since there is a specie-specific trend. For instance, fusiform species have more constrict lateral movement than vermiforms (i.e lamprey, eel and moray) and may require different inter-suture distances in order to optimize the healing time and suture maintenance.

Table 2. Wound healing, wound inflammation and suture integrity in goldfish (n = 4). A 36 mm incision was sutured using a simple-interrupted pattern at 3, 6 or 9 mm inter-suture-distance (ISD) and observed for wound healing, wound inflammation and suture integrity at 4 days intervals.

Days	Wound healing			Wound inflammation			Suture integrity		
	3 mm	6 mm	9 mm	3 mm	6 mm	9 mm	3 mm	6 mm	9 mm
0	5.00 ± 0.0Aa	5.00 ± 0.00Aa	5.00 ± 0.00Aa	0.0 ± 0.00aC	0.0 ± 0.00aC	0.0 ± 0.00aC	5.0 ± 0Aa	5.0 ± 0.00Aa	5.0 ± 0.00Aa
4	4.5 ± 0.57Aa	4.75 ± 0.50ABa	4.75 ± 0.50Aa	4.25 ± 0.95Aa	3.25 ± 1.26Aab	2.25 ± 0.50Ab	5.0 ± 0Aa	5.0 ± 0.00Aa	5.0 ± 0.00Aa
8	3.5 ± 0.57Aa	3.5 ± 0.57Ba	3.00 ± 0.82Ba	4.25 ± 0.95Aa	3.5 ± 1.00Aab	2.0 ± 0.00Ab	5.0 ± 0Aa	5.0 ± 0Aa	4.0 ± 1.15ABa
12	1.25 ± 1.50Ba	2.0 ± 1.41Ca	0.75 ± 1.50Cb	3.5 ± 1.3ABa	2.75 ± 0.5ABa	1.0 ± 0.00Bb	4.25 ± 0.5Aa	5.0 ± 0.00Aa	4.0 ± 1.15ABa
16	0.00 ± 0.00Ba	0.00 ± 0.00Da	0.00 ± 0.00Ca	2.5 ± 1ABa	1.5 ± 0.58BCab	0.75 ± 0.50Bb	2.75 ± 0.95Ba	3.25 ± 1.25Ba	3.5 ± 1.00ABa
20	0.00 ± 0.00Ba	0.00 ± 0.00Da	0.00 ± 0.00Ca	1.5 ± 0.58BCa	0.5 ± 0.58Cb	0.0 ± 0.00Cb	1.0 ± 0.00Ca	1.0 ± 0.80Ca	1.25 ± 2.50Ba

Different capital letters within columns and small letters within rows denotes no significantly differences by the Tukey test ($p < 0.05$).

Inflammation was more intensive at 6 and 3 mm as inter-suture distance, since the sutures increased the penetration of water containing microorganisms and the surface for its proliferation (WAGNER; DON STEVENS, 2000).

We did not observe any difference in suture integrity when comparing the treatments, but this parameter decreased with time. From the 16th day after suturing, significant decrease in suture integrity was observed in all treatments.

In the present study, no mortality was observed in any treatments. This result can be attributed to 1) the intrinsic rusticity of the specie, 2) the short period of handling, 3) the adoption of aseptic procedures and 4) post-operative care, as discussed by other authors (WAGNER et al., 1999; HARMS, 2005).

As observed in the results above, we conclude that 2-phenoxyethanol at 0.4‰ is effective as a maintenance dosage for the goldfish. In addition, an inter-suture distance of 9 mm optimizes the healing time, wound inflammation and suture integrity. These parameters have relevance for surgical procedures in the goldfish.

Acknowledgements

Authors are grateful to Alice Emi Imai for the English review of this article.

References

BANDILLA, M.; HAKALAHTI T.; HUDSON P. J.; VALTONEN, E. T. Aggregation of *Argulus coregoni* (Crustacea: Branchiura) on rainbow trout (*Oncorhynchus mykiss*): a consequence of host susceptibility or exposure? **Parasitology**, v. 130, n. 2, p. 169-176, 2005.

BART, A. N.; DUNHAM, R. A. Factors affecting survival of channel catfish after surgical removal of testes. **The Progressive Fish Culturist**, v. 52, n. 4, p. 241-246, 1990.

BERRY JR.; C. R.; BABEY, G. J.; SHRADER, T. Effect of *Lernaea cyprinacea* (Crustacea: Copepoda) on stocked rainbow trout (*Oncorhynchus mykiss*). **Journal of Wildlife Diseases**, v. 27, n. 2, p. 206-213, 1991.

CLOUD, J. G. Surgical transplantation of sexually immature ovaries of rainbow trout (*Oncorhynchus mykiss*). **Journal of Experimental Zoology**, v. 298, n. 1, p. 73-76, 2003.

COOKE, S. J.; CROSSIN, G. T.; PATTERSON, D. A.; ENGLISH, K. K.; HINCH, S. G.; YOUNG, J. L.; ALEXANNER, R. F.; HEALEY, M. C.; VAN DER KRAAK, G.; FARRELL, A. P. Coupling non-invasive physiological assessments with telemetry to understand inter-individual variation in behaviour and survivorship of sockeye salmon: development and validation of a technique. **Journal of Fish Biology**, v. 67, n. 5, p. 1342-1358, 2005.

HARMS, C. A. Surgery in fish research: common procedures and postoperative care. **Lab Animal**, v. 34, n. 1, p. 28-34, 2005.

HARMS, C. A.; LEWBART, G. A. Surgery in fish. **Veterinary Clinics of North America**, v. 3, n. 3, p. 724-759, 2000.

HILSDORF, A. CANEPPELE, D.; KRIEGER, J. E. Muscle biopsy technique for electrophoresis analysis of fish from the genus *Brycon*. **Genetics and Molecular Biology**, v. 22, n. 4, p. 547-550, 1999.

INOUE, L. A. K. A.; NETO, C. S.; MORAES, G. Standardization of 2-phenoxyethanol as anesthetic for juvenile *Brycon cephalus* (Gunther, 1869): the use in field procedures. **Ciência Rural**, v. 34, n. 2, p. 563-565, 2004.

JENKINS, N.; DODD, J. M. Effects of ovariectomy of the dogfish *Scyliorhinus canicula* L. on circulating levels of androgen and oestradiol and on pituitary gonadotrophin content. **Journal of Fish Biology**, v. 21, n. 3, p. 297-303, 1982.

KOBAYASHI, A.; SOHN, Y. C.; YOSHIURA, Y.; AINDA, K. Effects of sex steroids on the mRNA levels of gonadotropin subunits in juvenile and ovariectomized goldfish *Carassius auratus*. **Fisheries Science**, v. 66, n. 2, p. 223-231, 2000.

MOHAMED, S. J. Comparative efficacy of four anesthetics on common carp *Cyprinus carpio* L. **Acta Ichthyologica et Piscatoria**, v. 29, n. 2, p. 91-97, 1999.

MYLONAS, C. C.; CARDINALETTI , G.; SIGELAKI, I.; POLZONETTI-MAGNI, A. Comparative efficacy of clove oil and 2-phenoxyethanol as anesthetics in the aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) at different temperatures. **Aquaculture**, v. 246, n. 4, p. 467-481, 2005.

NAGLER, J. J.; CLOUD, J. G.; WHEELER, P. A.; THORGAARD, G. Testis transplantation in male rainbow trout (*Oncorhynchus mykiss*). **Biology of Reproduction**, v. 64, n. 2, p. 644-646, 2001.

PAUKERT, C. P.; CHVALA, P. J.; HEIKES, B. L.; BROWN, M. L. Effects of implanted transmitter size and surgery on. survival, growth, and wound healing of bluegill. **Transactions of the American Fisheries Society**, v. 130, n. 5, p. 975-980, 2001.

PETERSON, S. A.; SICKLE, J. VAN; HUGHES, R. M.; SCHACHER, J. A.; ECHOLS, S. F. A biopsy procedure for determining filet and predicting whole-fish mercury concentration. **Archives of Environmental Contamination and Toxicology**, v. 48, n. 1, p. 99-107, 2005.

ROSS, L. G.; GEDDES, J. A. Sedation of warm-water fish species in aquaculture research. **Aquaculture**, v. 16, n. 2, p. 183-186, 1979.

SCHNAIDER, T. B.; SOUZA, C. Aspectos éticos da experimentação animal. **Revista Brasileira de Anestesiologia**, v. 53, n. 2, p. 278-85, 2003.

SMITH, L. S.; BELL, G. R. Anesthetic and surgical techniques for Pacific salmon. **Journal of the Fisheries Research Board of Canada**, v. 24, n. 7, p. 1579-1588, 1967.

STOSKOPF, M. K. Surgery. In: STOSKOPF, M. K. (Ed.). **Fish medicine**. Phyladelphia: W.B Saunders, 1993. p. 91-97.

TEIXEIRA, A.; CORTEZ, R. M. V. PIT telemetry as a method to study the habitat requirements of fish populations: application to native and stocked trout movements. **Hydrobiologia**, v. 582, n. 1, p. 171-185, 2007.

WAGNER, G. N.; DON STEVENS, E. Effects of different surgical techniques: suture material and location of incision site on the behaviour of rainbow trout (*Oncorhynchus mykiss*). **Marine and Freshwater Behaviour and Physiology**, v. 33, n. 1, p. 103-14, 2000.

WAGNER, G. N.; DON STEVENS, E.; HARVEY-CLARK, C. Wound healing in rainbow trout following surgical preparation with a povidine-iodine antiseptic. **Journal of Aquatic Animal Health**, v. 11, n. 4, p. 373-382, 1999.

WAGNER, G. N.; DON STEVENS, E.; BYRNE, P. The effects of suture type and patterns on surgical wound healing in rainbow trout. **Transactions of the American Fisheries Society**, v. 129, n. 5, p. 1196-1205, 2000.

WOOSTER, G. A.; HSU, H. M.; BOWSER, P. R. Nonlethal surgical procedures for obtaining tissue samples for fish health inspections. **Journal of Aquatic Animal Health**, v. 5, n. 3, p. 157-164, 1993.

Received on February 6, 2008.
Accepted on June 10, 2008.