Evaluation of surgery procedures for implanting telemetry transmitters into the body cavity of tilapia *Oreochromis aureus*

Xavier Thoreau and Etienne Baras

University of Liège, Laboratory of Fish Demography and Aquaculture, 10, Chemin de la Justice, B-4500 Tihange, Belgium.
E-mail: E.Baras@ulg.ac.be

Received July 2, 1996; accepted April 9, 1997.

Thoreau X., E. Baras. *Aquat. Living Resour.*, 1997, **10**, 207-211.

Abstract

Surgery procedures were used to implant telemetry transmitters into the body cavity of adult (574 - 1 033 g) tilapias *Oreochromis aureus* in aquaculture tanks (4 m^2,1.5 m^3, 26.5 ± 0.5°C, ≥ 5.0 mg O_2 l^{-1}) and their effect on fish survival, growth and behaviour was evaluated. Only one out of 35 implanted fish died. With one exception, all fish consistently retained their transmitter until the end of the study (up to 30 months). Healing was faster when the incision was sutured with polyamide monofilament (5-14 days) than with other suture materials, due to tunnelling with atraumatic needles for catgut or fouling of braided silk. In all 10 fish sacrificed after 30 and 50 days, the transmitter had become encapsulated by connective tissue. No infection or damage to the viscera was observed.

The activity of four tilapias (903-1 033 g) equipped with motion sensitive transmitters was telemetered during the recovery from anaesthesia and surgical procedures. All four fish maintained a normal diurnal activity rhythm pattern throughout the study but had low levels of activity during the first 12-24 h. Based on the evolution of their resting posture after surgery, it is suggested that tilapias need 3 to 4 days to completely compensate the negative buoyancy resulting from anaesthesia and tagging.

Keywords: tagging, surgery, telemetry, fish, tilapia, tropical aquaculture.

Evaluation d'une procédure chirurgicale d'implantation abdominale d'émetteurs de biotélémétrie chez le tilapia Oreochromis aureus.

Résumé

Des émetteurs de biotélémétrie ont été implantés dans la cavité intra-péritonéale de tilapias *Oreochromis aureus* (574-1 033 g) en bassins d'aquaculture (4 m^2, 1,5 m^3, 26,5 ± 0,5°C, ≥ 5,0 mg O_2 l^{-1}). L'effet du marquage sur la rétention des marques, la survie, la croissance et le comportement des poissons a été étudié. Un seul des 35 poissons marqués est mort, et à une exception près, tous les tilapias marqués ont gardé leur émetteur jusqu'au terme de l'étude (30 mois). La cicatrisation était plus rapide lorsque l'incision était suturée avec du polyamide monofilament qu'avec d'autres matériels de suture, en raison des difficultés inhérentes à l'utilisation d'aiguilles ne provoquant pas de traumatisme (pour catgut) ou du développement d'algues sur la soie tressée. Trente et cinquante jours après l'opération, aucun dégât interne ou infection n'était observé chez 10 individus sacrifiés, dont l'émetteur était encapsulé dans une capsule de tissus conjonctifs.

Le comportement de quatre individus (903-1 033 g) équipés d'émetteurs de télémétrie à circuit d'activité a été enregistré pendant les phases de récupération de l'anesthésie et de la chirurgie. Les quatre tilapias ont maintenu un rythme d'activité diurne mais leur activité était nettement réduite pendant les 12-24 heures consécutives à l'opération. L'évolution de la posture de repos adoptée par les tilapias après la chirurgie indique qu'un délai de 3-4 jours est nécessaire à la récupération de l'équilibre et de la flottabilité négative induite par l'anesthésie et le marquage.

Mots-clés : marquage, chirurgie, télémétrie, poisson, tilapia, aquaculture tropicale.

Aquat. Living Resour. ISSN 0990-7740/97/04/$ 7.00/© IFREMER-Gauthier-Villars

INTRODUCTION

Monitoring the behaviour of fish inside their culture environment may represent an efficient and real time feedback of fish status, degradation of culture environment or adequacy of feeding schedules to fish activity or appetite (*e.g.* Kadri *et al.*, 1991; Juell, 1995). Available techniques include video analyses (Smith *et al.*, 1993), use of computerised self-feeding (Boujard *et al.*, 1993), of PIT-tag data entry stations (Brännas and Alanärä, 1993) and of telemetry techniques (Lagardère *et al.*, 1988). Underwater telemetry techniques offer a wide range of advantages (see Priede and Swift, 1992; Baras and Lagardère, 1995) but the attachment of telemetry transmitter often represents an invasive procedure which may affect the behaviour or performance of the fish to variable extents (see Summerfelt and Smith, 1990; Baras, 1991; Baras *et al.*, in press). Numerous biases reported in the literature are specific to the procedure and/or species tagged, and it can hardly be predicted how a species, which is tagged for the first time, will react to the tagging procedure. This feasibility study aimed to evaluate i) the adequacy of surgery techniques for tagging intensively cultured blue tilapia *Oreochromis aureus* with telemetry tags and ii) the duration and intensity of the post-tagging stress which condition the representativity and significance of telemetry records from probe fish.

MATERIAL AND METHODS

The fish used in these experiment were female blue tilapia *O. aureus* reared at 27 ± 0.5°C, ≥ 5 mg of O_2 l^{-1}, in 1.5 m^3 (4 m^2) indoor flow through tanks of the Tihange Fish Breeding Station of the University of Liège where the hot water supply originates from nuclear power plant effluents. The protocol used for fish surgery is inspired by these of Hart and Summerfelt (1975) and of Baras and Philippart (1989). Tilapias were anaesthetised in a 0.4 ml l^{-1} solution of 2-phenoxy-ethanol, which is a widely used anaesthetic for temperate and tropical fish species (Smit *et al.*, 1979; Moore *et al.*, 1994, Baras *et al.*, in press). Fish reached anaesthesia stage III.2 (*i.e.* no reaction to tactile or painful stimulus, no deliberate muscular movement other than ventilation; MacFarland and Klontz, 1969) after an average of 7.9 min (SD = 1.8 min) then were placed ventral side up into V-shaped support tailored to their morphology. A mid-ventral incision was made between the pelvic girdle and the anus, after the removal of a single row of scales. The length of the incision did not exceed twice the diameter of the transmitter in order to minimise the risk of expulsion through the incision zone before healing was completed. An epoxy dummy transmitter, similar in shape and weight to the model used for the behavioural study, was inserted cranially through an abdominal incision that was made on the mid ventral line, after the removal of the central row of scales. The incision was closed by three separate stitches, 8-9 mm apart. Due to the elasticity of cichloïd scales, a scale was removed at each site of needle passage. Three suture material were evaluated: plain catgut (SoftCatgut), braided silk (Ethicon™) and polyamide monofilament (Ethilon™) (details in Table 1).

On the average, the surgery was completed after 10 to 12 min. Fish were weighed to the nearest 0.5 g, tagged individually with passive integrated transponder (PIT) tags inserted into the dorsal musculature then allowed to recover for 30 min. They were stocked in a 4 m^2 (1.5 m^3) flow through tank, together with fish of the control group (20 fish PIT tagged at the same time) to test for their ability to compete for food (mean daily food ratio: 5 g $fish^{-1}$; distributed daily in a self-feeder). During the next 50 days, fish were controlled at regular intervals (around 5 days). Non absorbable suture materials were removed during the first control to avoid any risk of scale abrasion, skin irritation or cutting. We measured weight variations, the time for complete healing of the incision zone and sites where suture needles penetrated the body wall. Tag loss was checked empirically by search for dummies on the bottom of the empty tank after each control. Survival was checked daily. Thirty and 50 days after surgery, five

Table 1. – Effect of suture material on survival, transmitter retention and healing of surgically implanted tilapia *O. aureus*. The calculation of tag retention at 50 days and 30 months is based on surviving fish only.

	SoftCatgut 3/0	Ethicon 3/0™	Ethilon 3/0™
Suture material	Plain Catgut	Braided silk	Polyamide monofilament
Resorbable	YES	NO	NO
Needle: section	round (atraumatic)	triangular (cutting)	triangular (cutting)
length	18 mm	16 mm	19 mm
curvature	3/8	3/8	3/8
Number of fish	5	5	25
Survival (%) 1-50 days	100.0	100.0	96.0
50 days-30 months	100.0	100.0	100.0
Tag retention 1- 50 days (%)	100.0	100.0	95.8
Tag retention 50 days - 30 months	100.0	100.0	100.0
Healing (days)			
incision zone: mean ± SD (min-max)	9.4 ± 4.4 (5-15)	18.8 ± 3.7 (15-25)	10.0 ± 2.8 (5-14)
needle passage: mean ± SD (min-max)	25.2 ± 12.5 (12-43)	22.2 ± 6.3 (11-25)	10.8 ± 4.5 (5-26)

fish were sacrificed to evaluate the possible damages to the viscera, the healing of the midventral body wall (thickness, nearest 0.05 mm) and to check for any risk of encapsulation by connective tissue or by the intestine.

In order to provide insights on the behaviour of tilapia during the recovery from surgery, four tilapias (903-1 033 g) were tagged with motion sensitive 40 MHz radio transmitters (Advanced Telemetry Systems - ATS, Inc., 70 mm long, 12 mm in diameter; 15 g, using polyamide filaments to close the incision. Each transmitter was implanted in order to switch to high pulse rate (84 ± 1 pulse per min) when the posture of the fish was horizontal or bended forward. Activity and resting postures were discriminated from the continuous recording of transmitter pulse rate and signal strength variations by a Lotek SRX-400 receiver equipped with W-18 processor (two fish telemetered simultaneously; see details on signal recording and processing in Baras, 1996). Activity budgets were defined as the ratio (%) of the running duration of activity records on total record time at the hourly, periodic (diurnal or nocturnal) and daily (24-h) scales. The recovery from the negative buoyancy induced by anaesthesia and tag insertion was investigated through a finer analysis of resting posture. The visual observation of naive fish (10 fish in large aquaria; 4 hours per fish, day and night) indicate that tilapia most frequently ≥ 75% rest in the middle part of the water column, in an oblique posture with their snout heading towards the surface of the water. With respect to our standardised implantation mode, this posture corresponded to low pulse rate whereas the horizontal posture - resulting from the non compensated transmitter insertion in front of the centre of gravity of the fish - corresponded to a high pulse rate (84 ± 1 pulse per min). The proportion of rest in an oblique posture by day and by night was used as an index of recovery of negative buoyancy.

RESULTS

Only one of 35 implanted fish - as well as one from the control group - died during the experiments (χ^2 = 0.17; df = 1; $p > 0.10$), for reasons independent from surgery and handling (*i.e.* leaping out of the tank). On two occasions, we observed a loop of the intestine protruding out of the intraperitoneal cavity due to loose sutures but no mortality or infection was noted after an additional stitch was made. One fish (sutured with braided silk) lost its transmitter through the incision less than 24 hours after surgery, due to loose stitches placed 10-12 mm apart.

All fish completely healed the incision in less than four weeks (Table 1) but the healing rate was proportionally faster when fish had been sutured with polyamide or catgut filaments than with braided silk (Kruskal-Wallis H = 12.4; df = 34; $p < 0.01$). Small cuts and wounds were frequently observed at sites of needle passage, presumably as a consequence of the presence of permanent suture material at places through tissues uncovered with scales. These wounds healed progressively after suture removal. The healing time was significantly shorter with polyamide monofilament than with plain catgut or braided silk (Kruskal-Wallis H = 13.3; df = 34; $p < 0.01$). All surgically tagged fish lost weight during the first 10 days after surgery, then progressively regained weight. The weight loss comparatively to the control group was significant at the 0.05 level for fish sutured with catgut or braided silk, but not for fish sutured with polyamide (respective mean weight losses: 2.6, 5.7 and 0.9 % after five days, 3.5, 3.3 and 1.8 % after 10 days).

All 10 fish sacrificed after 30 and 50 days had retained their transmitter. Connective tissue had proliferated around the incision zone and replaced the epithelium and mid ventral muscular layers. After 30 days, the thickness of the muscular layer was decreased by 0.27 mm on the average (0.10-0.35 mm). After 50 days, this deficit was reduced to 0.16 mm (0.10-0.30 mm). No inflammation or infection was observed and neither the gut nor the gonads had been damaged by the transmitter. In each fish, the transmitter was encapsulated by a fibrous capsule - precisely at the site where it was implanted - thus limiting its mobility and subsequent risk of internal damage. Similar observations were made in the remaining fish, that were sacrificed 30 months after surgery.

All four telemetered fish showed similar behavioural responses. The activity during the first night (68-69 %) was less than during the following nights (75-96 %), except for fish 1 which maintained low nocturnal activity levels till the third night (Fig. 1). The activity rhythm though consistently remained diurnal in all four fish. Diurnal activity levelled around 90-95 % on the third day after surgery, suggesting the recovery of normal activity.

During the first night after surgery, all tilapia showed an abnormally high proportion of rest in horizontal posture (33-96 % against less than 25 % in non

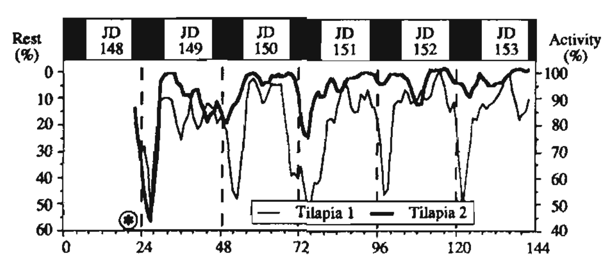

Figure 1. – Activity rhythm pattern of two cultured tilapia *O. aureus* during postsurgery period (26.5 ± 0.5°C, 1.5 m^2 flow through tank, diurnal feeding). Activity as hourly means. * surgical implantation. JD = Julian days. Light bars: day; dark bars: night. Fish 1: 22 950 records; fish 2: 22 091 records.

implanted fish). The normal ratio was recovered after 72 hours and maintained later on (Fig. 2). During the following days, no significant deviation of activity budget or resting posture was observed comparatively to days 3 and 4 (ANOVA; $p \geq 0.10$).

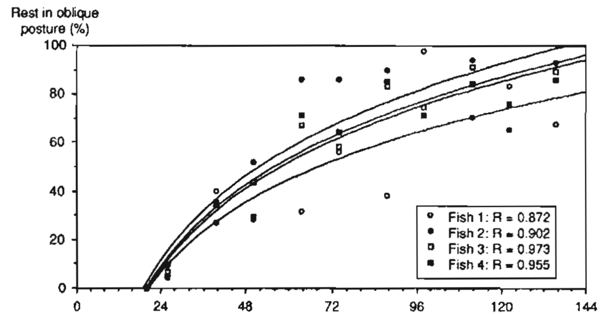

Figure 2. – Recovery from surgery induced negative buoyancy in tilapia *O. aureus*, using the proportion of rest in an oblique posture - by day and by night - as a recovery index (semi-log curve fits). Normal proportion of rest in oblique posture ≥ 75%. Records from 4 telemetered fish stocked in a 1.5 m^2 flow through tank (26.5 ± 0.5°C).

DISCUSSION

Our results showed the adequacy of surgical implantation procedures for attaching transmitters to tilapia *Oreochromis aureus* as only one fish died for reasons independent of surgery. No infection was observed despite no prophylactic treatment was used on fish stocked at high temperature (27 °C). Lucas (1989) and Baras (1992) reported similar success in rainbow trout *Oncorhynchus mykiss* and *Barbus barbus* with or without post-operative care. The high survival rate can be related to the fast healing process (on the average, 10 days when using polyamide monofilament stitches) which is much faster than for cold-temperate (Pedersen and Andersen, 1985; Lucas, 1989; Baras, 1992; Moore *et al.*, 1994) or temperate-tropical fish species (Ross and Kleiner, 1982; Schramm and Black, 1984; Marty and Summerfelt, 1986). Fast healing also presumably limited the risks of transmitter expulsion through the incision. The encapsulation of the transmitter by host tissue within the first month after surgery minimised the probability of movement inside the body cavity and presumably limited the risks of damaging the viscera or eroding the body wall. Obviously, the encapsulation process was not prone to cause a transintestinal expulsion process (Marty and Summerfelt, 1986) as all fish retained their transmitters in the long run (30 months).

With respect to suture material, this study further confirmed the adequacy of catgut filament for external suturing in fish (Baras, 1992; Gilliland, 1994). However, the atraumatic needles used with catgut filaments could not be easily passed through the very strong body wall of tilapia. This resulted into larger tunnels at needle passage sites than with the cutting needles used with non absorbable filaments, and longer healing time. The healing of both incision and needle passage sites was substantially longer with braided silk than with polyamide monofilament, despite both filaments were set on similar cutting needles. When non absorbable filaments were removed on the first control of fish, we observed some fouling and traces of fish bites on the braided silk filaments. These traces suggest some form of grazing activity by other fish on the algae, that would have caused additional tensions on wounded tissues and interfered with tissue reconstruction. The better overall adequacy of polyamide suture material set on cutting needle was confirmed by the changes in fish body weight throughout the 50-day experiment, since weight losses were non significant with respect to the control group.

The diurnal activity rhythm pattern normally observed in tilapia (Mélard, 1986; Ross and McKinney, 1988) was not disrupted by the implantation process and was consistently maintained throughout the two week experiment. Activity was though slightly depressed on the first night after tagging but to a lesser extent than usually reported by authors mentioning a post-tagging hypo-activity (*e.g. O. mykiss* does not feed during the first two days following tagging; Lucas, 1989). Based on resting posture, it is suggested that the short-term hypo-activity in tilapia originates from the need to progressively compensate the negative buoyancy induced by a depressed swim bladder and weight added (Gallepp and Magnuson, 1972; Fried *et al.*, 1976). The postural disequilibrium was not fully recovered before 3 days presumably because tilapia are physoclist fish and can not quickly refill their swimbladder by gulping air at the surface. Long term effects of surgery on behaviour were restricted to slight differences between individual activity budgets though such differences may genuinely reflect different physiological status with respect to maturation stage. These differences were levelled after 10-12 days, *i.e.* a delay similar to the time for healing.

In conclusion, the results clearly indicate that the surgical implantation procedure is adequate for tilapias *O. aureus*. However, any confident use of telemetered fish for biomonitoring purposes should be delayed by at least 3 to 4 days after surgery, and presumably by up to two weeks, when the fish has recovered its physical integrity. This should be taken into account when programming intelligent transmitters with activation delays. It may be argued that healthy control fish possibly impaired the recovery of tagged fish and presumably made it longer than if tagged fish had been reared separately. However, there is no clearcut evidence that it really happened and that this impairement would have outweighed the one originating from rearing territorial fish at low density.

Acknowledgements

The authors wish to thank Dr. C. Mélard and Mr. D. Desprez (ARDA) for constructive comments on the biology of tilapias. We are indebted to the Ministry of Technologies of the Walloon Region for supporting the telemetry research programme (FIRST-University programme, University of Liège) and to Electrabel S. A. for supporting the tropical fish aquaculture programme. X. Thoreau received a COMETT grant at the time of the study.

REFERENCES

Baras E. 1991. A bibliography on underwater telemetry. *Can. Rep. Fish. Aquat. Sci.* **1819**, 55 p.

Baras E. 1992. Etude des stratégies d'occupation du temps et de l'espace chez le barbeau fluviatile, *Barbus barbus* (L.). *Cah. Ethol.* **12**, 125-442.

Baras E. 1996. Photoperiod mediated variations of daily activity budgets in cultured tilapias *Oreochromis aureus*. *In:* Underwater biotelemetry, E. Baras, J.C. Philippart eds. University of Liège, Belgium, 187-194.

Baras E., J.P. Lagardère 1995. Fish telemetry in aquaculture: review and perspectives. *Aquacult. Int.* **3**, 77-102.

Baras E., J.C. Philippart 1989. Application du radio-pistage à l'étude éco-ethologique du barbeau fluviatile (*Barbus barbus* L.). Problèmes, stratégies et premiers résultats. *Cah. Ethol. Appl.* **9**, 467-494.

Baras E., C. Birtles, L. Westerloppe, X. Thoreau, M. Ovidio, D. Jeandrain, J.C. Philippart (in press). A critical review of surgery techniques for implanting telemetry devices into the body cavity of fish. *In*: Proc. 5th European Conference on Wildlife Telemetry. Y. Le Maho ed. Strasbourg, France, 26-30 August 1996, 10 p.

Boujard T., S. Brett, L. Lin, J.F. Leatherland 1993. Effect of restricted access to demand feeder on diurnal pattern of liver composition, plasma metabolites and hormone levels in *Oncorhynchus mykiss*. *Fish Physiol. Biochem.* **11**, 337-344.

Brännäs E., A. Alanärä 1993. Monitoring the feeding activity of individual fish with a demand feeding system. *J. Fish Biol.* **42**, 209-215.

Fried S.M., J.D. McCleave, K.A. Stred 1976. Buoyancy compensation by Atlantic salmon (*Salmo salar*) smolts tagged internally with dummy telemetry transmitters. *J. Fish. Res. Board Can.* **33**, 1377-1380.

Gallepp G.W., J.J. Magnuson 1972. Effects of negative buoyancy on the behavior of the bluegill, *Lepomis macrochirus* Rafinesque. *Trans. Am. Fish. Soc.* **101**, 507-512.

Gilliland E.R. 1994. Comparison of absorbable sutures used in largemouth bass liver biopsy surgery. *Progress. Fish. Cult.* **56**, 60-61.

Hart L.G., R.C. Summerfelt 1975. Surgical procedures for implanting ultrasonic transmitters into flathead catfish (*Pylodictis olivaris*). *Trans. Am. Fish. Soc.* **104**, 56-59.

Juell J.-E. 1995. The behaviour of Atlantic salmon in relation to efficient cage-rearing. *Rev. Biol. Fish.* **5**, 320-335.

Kadri S., N.B. Metcalfe, F.A. Huntingford, J.E. Thorpe 1991. Daily feeding rhythms in Atlantic salmon in sea cages. *Aquaculture* **92**, 219-224.

Lagardère J.P., J.J. Ducamp, L. Frikha, M. Spérandio 1988. Ultrasonic tracking of common sole (*Solea vulgaris* Quensel, 1806) in a saltmarsh: methods and fish response to some environmental factors. *J. Appl. Ichthyol.* **4**, 87-96.

Lucas M.C. 1989. Effects of implanted dummy transmitters on mortality, growth and tissue reaction in rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.* **35**, 577-587.

MacFarland W.N., G.W. Klontz 1969. Anesthesia in fishes. *Federal Proceedings* **28**, 1535-1540.

Marty G.D., R.C. Summerfelt 1986. Pathways and mechanisms for expulsion of surgically implanted dummy transmitters from channel catfish. *Trans. Am. Fish. Soc.* **115**, 577-589.

Mélard Ch. 1986. Les bases biologiques de l'élevage intensif du tilapia du Nil. *Cah. Ethol. Appl.* **6**, 1-224.

Moore A., G.D. Pickett, D.R. Eaton 1994. A preliminary study on the use of acoustic transmitters for tracking juvenile bass (*Dicentrarchus labrax*) in an estuary. *J. Mar. Biol. Assoc. U.K.* **74**, 451-454.

Pedersen B.H., N.G. Andersen 1985. A surgical method for implanting transmitters with sensors into the body cavity of the cod (*Gadus morhua* L.). *Dana* **5**, 55-62.

Priede I.G., S.M. Swift eds. 1992. Wildlife biotelemetry: remote monitoring and tracking of animals. Ellis Horwood, Chichester, UK, 708 p.

Ross M.J., C.F. Kleiner 1982. Shielded needle technique for surgically implanting radio frequency transmitters in fish. *Progress. Fish Cult.* **44**, 41-43.

Ross L.G., R.W. McKinney 1988. Respiratory cycles in *Oreochromis niloticus* (L.), measured using a six-channel microcomputer-operated respirometer. *Comp. Biochem. Physiol.* **89**, 637-643.

Schramm H.L. Jr., D.J. Black 1984. Anaesthesia and surgical procedures for implanting radio transmitters into grass carp. *Progress. Fish Cult.* **46**, 185-190.

Smit G.L., J. Hattingh, A.P. Burger 1979. Haematological assessment of the anesthetic MS_{222} in natural and neutral form in three freshwater fish species: intraspecies differences. *J. Fish Biol.* **15**, 645-653.

Smith I.P., N.B. Metcalfe, F.A. Huntingford, S. Kadri 1993. Daily and seasonal patterns in the feeding behaviour of Atlantic salmon (*Salmo salar* L.) in a sea cage. *Aquaculture* **117**, 165-178.

Summerfelt R.C., L.S. Smith 1990. Anesthesia, surgery, and related techniques. *In*: Methods for fish biology. C.B. Schreck, P.B. Moyle eds. American Fisheries Society, Bethesda, USA, 213-272.