

Photo-identification and the effects of tagging on the Patagonian catfish *Hatcheria macraei*

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Received: 13 March 2014 / Accepted: 22 September 2014 / Published online: 27 September 2014
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Abstract Effects of subcutaneous visible implanted alphanumeric (VIA) microtags, pelvic fin excision, and individual photo-identification (Pid) based on natural spot patterns were experimentally evaluated in the small stream benthic Patagonian catfish *Hatcheria macraei*. VIA tag retention was 90 % during the first 45 days, decreasing to 80 % at day 200, and 66 % at the end of the experiment, at day 254. Fin regeneration was not evident during the experiment. Survival, growth rate or condition factor (Fulton's K) did not differ between tagged or pelvic fin clipped catfish and a control group. Spot patterns varied greatly among individuals and remained constant throughout the experimental period, thereby making identification of individual Pid possible in *H. macraei*. Tagging was not effective in identifying individual fish due to mark loss or difficulty in reading VIA tag codes. The combination of Pid with VIA tagging or fin clipping techniques in longterm experiments is advisable in order to facilitate individual recognition. The Pid analysis described in this study is a low-cost

method that could potentially be applied to any fish with a variable spot pattern.

Keywords Fulton's K · Growth · Individual identification · Mark-recapture · Survival · Tag retention

Introduction

Identification of individual fish is often required in studies of life-history, age validation, stock status, behavior, migration, distribution, success of stocking programs, etc. (Phelps and Rodriguez 2011; Marshall and Pierce 2012). There are basically 2 animal identification techniques: invasive and non-invasive methods. The former implies the use of marks or tags, and is widely applied in ecology and management studies (Lucas and Baras 2000). These experiments must meet some basic requirements in order to obtain reliable population parameters (Williams et al. 2002; Amstrup et al. 2005); for example, marks must not alter fish behaviour, growth, or survival (Jepsen et al. 2008), and tag or mark loss must be minimal or known (Cowen et al. 2009). On the other hand, non-invasive methods such as photo-identification (Pid) have no deleterious effects on individuals, but can only be performed in species with natural features or marking (Martin-Smith 2011; Kitchen-Wheeler et al. 2012).

Skin pigmentation patterns have frequently been used to track individuals of diverse fish species, e.g. barracudas (Wilson et al. 2006), seadragons (Martin-Smith 2011), salmon (Merz et al. 2012) and sharks (Barker and Williams 2010). The arrangement of

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melanophores into macroscopic configurations, such as stripes or spots, may remain only during early life periods (e.g. Donaghy et al. 2005) or for many years, probably until death, in long-living species (Bansemer and Bennett 2008).

The small benthic catfish *Hatcheria macraei* (Girard, 1855) is a rheophilic and negatively phototactic (Menni 2004) species of the family Trichomycteridae that lives in cold, well-oxygenated waters (Ringuelet et al. 1967). The diurnal microhabitat use of *H. macraei* is associated with large substrate sizes with conspicuous interstitial space (Barriga et al. 2013). Arratia and Menu-Marque (1981) described a size-related habitat preference, mostly associated with the type of substrate and water depth. In addition, Barriga and Battini (2009) determined the indirect ontogeny (sensu Balon 1990) of this species and related its morphological constraints to habitat and feeding preferences. Furthermore, *H. macraei* presents an extremely wide variation in its spotted pattern (Arratia and Menu-Marque 1981); therefore, this species is an excellent candidate for the evaluation of Pid techniques based on spot pattern configuration.

Hatcheria macraei is widely distributed in low-order rivers of Patagonia (Unmack et al. 2009, 2012), in contrast with other native fishes [e.g. creole perch, *Percichthys trucha* (Valenciennes, 1833), Patagonian silverside, *Odontesthes hatcheri* (Eigenmann, 1909), or the small puyen, *Galaxias maculatus* (Jenyns, 1842)], which inhabit mostly lentic (lakes and reservoirs) or high-order lotic environments. The main goal of this study was to develop an individual identification technique to be applied in *H. macraei* population studies. The specific aims were to i) estimate tag retention rates and the effect of implanted tags on growth and survival, ii) evaluate the effect of pelvic fin excision on growth and survival, as well as time of fin regeneration in this species, and iii) evaluate temporal variation in melanophore spot patterns on the flanks of *H. macraei* as a potential Pid tool.

Materials and methods

Fish collection

Hatcheria macraei individuals were captured in the Pichileufu River, Río Negro province, Argentina (41°05'24" S, 70°49'42" W, 926 m a.s.l.) using a 24 V DC backpack electrofishing unit, model 12-B (Smith-Root Inc., Vancouver, WA, USA) and hand nets.

Fishing was performed mainly in riffles in an upstream direction on March 18th 2011. Fish captured ($n=131$), ranged from 45 mm to 120 mm total length (TL) including juveniles and adults, were transported to the *Centro de Salmonicultura Bariloche* of the *Universidad Nacional del Comahue* in San Carlos de Bariloche city, Río Negro province, Argentina.

Experimental design of trials

Fish were placed in 4 circular tanks of 100 l with a continuous water supply. As *H. macraei* is a negatively phototactic fish and remains hidden during daytime, the circular tanks were prepared with cobble substrate as shelter. After individuals allowed to acclimate for one week to the experimental conditions, all catfish were anaesthetized using benzocaine (0.02 g l^{-1}) and randomly assigned to one of four groups. Individuals in the first group ($n=37$) were marked with small ($1.2 \text{ mm} \times 2.7 \text{ mm}$) alphanumeric code tags (Northwest Marine Technology Inc., Shaw Island, WA, USA). These visible implant alphanumeric (VIA) tags were placed under the skin with a handheld injector. Owing to the small size of this species, up to 120 mm TL in the Pichileufu population, the chosen implantation area of tags was in the caudal peduncle flanks. Individuals in the second group consisted in animals previously marked with VIA tags ($n=13$) on different dates, released in the Pichileufu River, and recaptured on 18 March 2011 (Table 1). This group was used to test whether marked fish presented the same probability of survival as recently marked fish (i.e. group one). Fish belonging to the third group ($n=40$) were marked by clipping on the left pelvic fin. The final group ($n=41$), or control group, was made up of unmarked fish. After the group designation of individuals, each fish was photographed laterally, on its left side, with a digital camera. The focal distance was kept at a constant 20 cm during the experiment using a stative. A ruler was placed near the fish as a reference for the digital measurement of fish size (nearest 1 mm) in each picture. Following this, catfish were weighed to the nearest 0.01 g using an electronic scale (Scout Pro 400, Ohaus Corp., Florham Park, NY, USA) and were finally returned to their respective circular tank to recuperate. This procedure of anesthetization, photographing and weighing was repeated every two weeks for the first 4 months and monthly for the following four months. A total of 14 sessions were performed during the 254-day experiment.

Fish were live prey (*Tubifex* sp.) fed *ad libitum* every 2 or 3 days throughout the experiment. Even though all circular tanks were maintained in a similar way, fish were randomly distributed in the four circular tanks to avoid bias owing to eventual differences between tanks. Water temperature was registered each 30 min during the experiment using a data logger HOBO® Pendant (Onset, Cape cod, MA, USA) with an accuracy of 0.54 °C.

Tag retention and fin regeneration

VIA tag retention and pelvic fin regeneration were visually evaluated after fish sedation, from the second to the final session. Only fish without a tag were carefully examined. First, pelvic fins were inspected to identify individuals with clipped fins. Following this, individuals with intact fins were checked, looking for possible scars caused by losing the tags. Individual identification of all fish, including those with legible tag codes, was performed by Pid based on spot patterns (see Photo-identification analysis).

Growth, condition and survival

Growth was evaluated by measuring changes in both TL and total wet body mass (M) throughout the duration of the experiment. Somatic condition was also assessed using Fulton’s condition factor, $K = M \text{ TL}^{-3} 10^5$, where M is the total wet body mass (in g) and TL is the total length (in mm). Differences between groups throughout the experiment regarding TL, M and K were tested by using a repeated measures analysis of variance (ANOVA) (McCullagh and Nelder 1989). Each repeated measure corresponded to a session of data recording (i.e. fourteen dates).

Table 1 Number of specimens of *Hatcheria macraei* and date captured, marked with visible implant alphanumeric (VIA) tags, and released (i.e. mark date), and time elapsed till recapture (18 March 2011) in the Pichileufu River, Argentina. These fish formed the second group in the experimental trials

Mark date	Days elapsed to the recapture date	n
1 December 2010	107	2
15 December 2010	93	1
15 February 2011	31	2
21 February 2011	25	3
3 March 2011	15	3
10 March 2011	8	2

Life tables were constructed from survival data for each experimental group. Survival analysis was performed using the Kaplan-Meier method (Kaplan and Meier 1958). A Wilcoxon chi-square (χ^2) test was used to analyze cumulative survivorship in experimental groups. To test the influence of TL or M on the survival model, a Cox regression was performed using these variables as covariates.

Photo-identification analysis

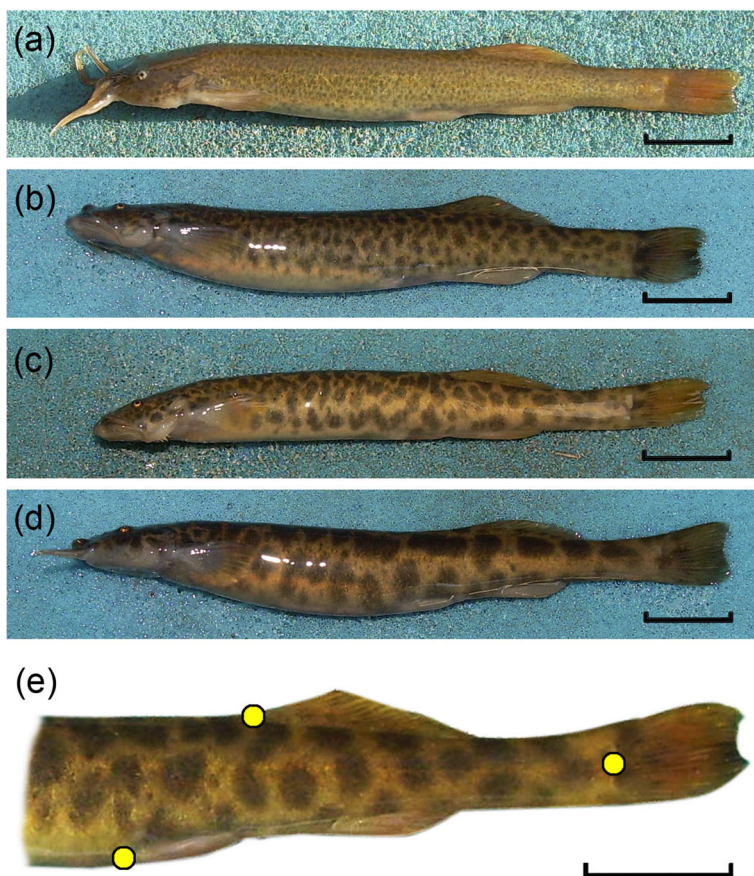
Spot varies widely in shape, size, density and position on the body of *H. macraei* (Fig. 1). Spot range from large black spots of different sizes and shapes to tiny dots uniformly distributed on the body, except for the ventral zone. The first and second groups (i.e. only marked individuals) were used to test whether the spot pattern could be used as an identification fingerprint. The image database generated over the 14 dates allowed the evaluation of temporal variation of the pigmentation pattern. The software I³S Manta v. 2.1 (Speed et al. 2007), freely available at <http://www.reijns.com>, was used to compare spot patterns from 4-megapixel pictures of each individual at t₀ (time at the beginning of the experiment) with subsequent photographic sessions. The software, created to identify individual manta rays [*Manta birostris* (Walbaum, 1792) and *Manta alfredi* (Kreffl, 1868)], is based on the differences in spot patterns between individuals. This software takes into account the number of spots, their shapes, and their position relative to three reference points. Spots and reference points are marked on each photograph manually. The structures taken as reference points in *H. macraei* were the origin of the dorsal fin, the origin of pelvic fins and the beginning of the caudal fin. Only spots delimited within these points were used as the fingerprint pattern (Fig. 1e). The program compares the unidentified fish image against the complete database of known individuals and generates a ranking of probability using an information criterion algorithm (Speed et al. 2007).

Results

Tag retention and fin regeneration

Inflammation was sometimes observed in the area where tags were inserted. In the cases where the tag had been lost, the insertion site had healed, leaving a

Fig. 1 Examples of spot pattern variation in *Hatcheria macraei* from small to larger spots (a - d). The caudal detail (e) shows the three fixed reference points (dots) taken in the use of software I³S Manta. Bars represent 1 cm



scar indicating where the tag had been located. As it was difficult to implant the tags in zones without pigmentation, independently of the particular individual spot pattern, the reading of VIA tag codes was almost impossible.

VIA tag retention was 90 % up to day 45, but then decreased to 80 % at day 200, and was 66 % at day 254 (Fig. 2a). There was no relationship between fish size (ranges every 5 mm TL) and percentage of tag retention (Spearman, $\rho = -0.149$, $n = 10$, $P = 0.66$).

Fin regeneration was not evident even at the end of the experiment; consequently, there was no misidentification between fin-clipped individuals and the control group.

Growth, condition and survival

The monthly growth rate was very slow in *H. macraei*: about 0.1 mm month⁻¹ in length and 0.05 g month⁻¹ in weight for each experimental group (Table 2). The *K* values increased up to day 100, then decreased until day 163, and finally stabilized towards the end of the

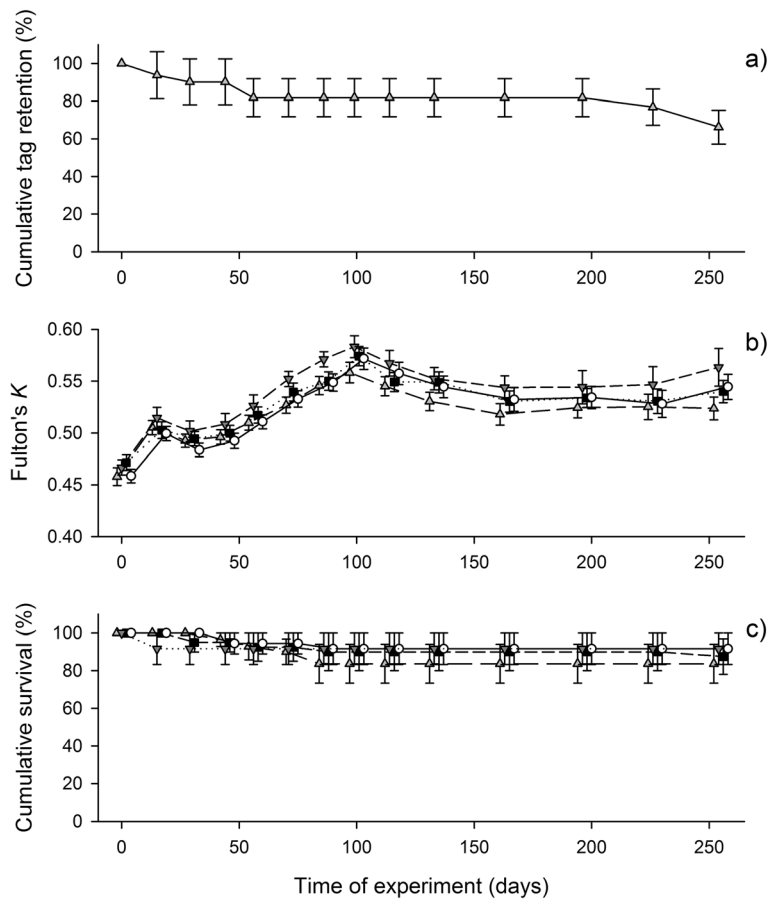
experiment (Fig. 2b). There were no differences between groups in the relative growth rate in TL (ANOVA, $F_{3, 113} = 2.08$, $P = 0.11$), M (ANOVA, $F_{3, 113} = 2.09$, $P = 0.11$), or in *K* (ANOVA, $F_{3, 113} = 0.72$, $P = 0.54$).

Overall survival decreased to 90 % at day 86, thereafter remaining constant till the end of the experiment. No differences in survival rate between groups (Wilcoxon, $\chi^2 = 0.92$, d.f. = 3, $P = 0.82$) were found (Fig. 2c). There was no relationship between TL or M of individuals and their survival (Cox regression, $P = 0.082$ and $P = 0.098$, respectively).

Photo identification analysis

Spot patterns varied greatly between individuals and differences remained constant throughout the experimental period in each individual (Fig. 3). A total of 43 out of 45 individuals (96 %) were correctly classified within the first position of the ranking generated by the software I³S Manta. Only two individuals were misclassified owing to their peculiar pigmentation

Fig. 2 **a** Cumulative VIA tag retention (Mean±SD), **b** Fulton's condition factor (mean±SE, $K = M TL^{-3} 10^5$), and **c** Mean (±SE) cumulative survival for *Hatcheria macraei* over 254 days of the experiment. Symbols correspond to four experimental groups: marked (△), recaptured (▽), fin excised (■), and control (○)



pattern. These catfish had very small, uniformly distributed dots on their flanks (Fig. 1a), which made delimitation of these dots extremely difficult. Despite this, all catfish were accurately identified within the fifth positions of the ranking generated by the programme. When only the largest spots within the delimited area were taken into account all individuals were correctly identified using I³S Manta.

Discussion

This is the first study to evaluate the use of fish tags and a Pid procedure to perform mark-recapture studies in the Patagonian catfish, *H. macraei*. The importance of population studies on this species lies not only in the knowledge of its life history and ecology, but also in the understanding of the invasive salmonid effect on native fish populations. The detrimental effect of salmonids on native species in rivers of low order in Patagonia (e.g. on

galaxiid species; Habit et al. 2010) could have been better tolerated by *H. macraei*, judging by their current distribution (Unmack et al. 2009, 2012) and density (Barriga et al. 2013).

In some individuals of *H. macraei* inflammation was observed in the area where tags were inserted, or the insertion site could be recognized by the lesion or scar it left. VIA tag retention depends on several factors, such as fish species, size, and tag location. In general, tag retention is lower in smaller individuals than in larger ones (Table 3). However we did not find any association between retention time and *H. macraei* size. Thus, this method is not biased by size in this species, at least in the size range analyzed.

The election of VIA tag location is mostly associated with rigid motionless structures covered by transparent skin, such as the sector between eyes and the opercular zone (Buckley et al. 1994; Crook and White 1995; Shepard et al. 1996; Hughes et al. 2000; Rikardsen et al. 2002; Summers et al. 2006), jaws (Olsen et al. 2004;

Table 2 Monthly growth rate (mean±SD) in length (TL, mm) and weight (M, g) of experimental group of *Hatcheria macraei*. Range at the beginning of the experiment (t_0) and sample size (n) are indicated

Group	Growth rate		Range at t_0		n
	mm month ⁻¹	g month ⁻¹	TL	M	
marked	0.065±0.065	0.028±0.040	45.8–95.1	0.47–3.82	32
recaptured	0.122±0.106	0.049±0.041	51.2–95.1	0.66–3.96	12
fin excised	0.084±0.076	0.040±0.046	54.8–106.6	0.84–4.90	35
control	0.090±0.095	0.045±0.072	49.8–120.1	0.48–7.57	38

Meerbeek et al. 2013) or nape (Griffiths 2002). These zones in *H. macraei* were not used for tag insertion owing to their small size and the unsuccessful results found in pilot experiments (J. P. Barriga, pers. comm.). In contrast, the caudal peduncle presented a large surface for the insertion of the VIA tag. However, the main inconvenience of this zone is that movement during swimming activity increases the chance of tag loss, and this could be the reason for low long-term retention in *H. macraei* compared to other species (Table 3).

Implanted tags could be used in short-term studies (i.e. less than 45 days). Although in this study individuals of *H. macraei* were marked, released and recaptured in their own habitat during periods longer than 45 days, this technique is not recommended for long-term population studies. The other disadvantage of these tags was associated with the difficulty of reading the tag codes because of the high degree of pigmentation found in this species. On the other hand, pelvic fin excision was detectable till the end of the experiment,

Fig. 3 Persistence of the spot pattern in *Hatcheria macraei* during the experimental period. Individual 1, (a) 19 April and (b) 31 August; individual 2, (c) 5 April and (d) 31 August, and individual 3, (e) 19 April and (f) 2 November. Bars represent 1 cm



Table 3 Comparison of visible implant alphanumeric (VIA) tag retention in different species of fishes

Species	Fish size (mm) ^a	Experimental period (days)	Tag dimensions (mm)	Tag retention (%)	reference
<i>Bathygobius cocosensis</i>	43–64 TL	90	2.5 × 1	77±19	Griffiths 2002
<i>Gadus morhua</i>	82–141 TL	150	2.5 × 1	67	Olsen et al. 2004
<i>Galaxias truttaceus</i>	> 90 TL	131	2.5 × 0.9	92	Crook and White 1995
<i>Girella elevata</i>	60–148 TL	90	2.5 × 1	32±20	Griffiths 2002
<i>Hippocampus abdominalis</i>	169±1 SL	90	2.5 × 1	100	Woods 2005
<i>Ictalurus punctatus</i>	280–379 TL	172	2.5 × 1	0	Buckmeier and Irwin 2000
<i>Oncorhynchus clarki lewisi</i>	100–324 FL	360	2.5 × 1	58	Shepard et al. 1996
<i>Ophiodon elongatus</i>	152–190 TL	160	3.0 × 1	100	Buckley et al. 1994
<i>Salmo trutta</i>	> 200 FL	180	2.5 × 1	42–97	Summers et al. 2006
<i>Salvelinus alpinus alpinus</i>	170–209 TL	160	2.5 × 1	78	Rikardsen et al. 2002
<i>Salvelinus fontinalis</i>	< 400 TL	100	2.5 × 1	89–97	Hughes et al. 2000
	< 400 TL	100	3.5 × 1.5	63–86	
<i>Sander vitreus</i>	553.7±4.1 TL	1825	3.5 × 1.5	76–77	Meerbeek et al. 2013
<i>Sebastes emphaeus</i>	–	330	1.5 × 0.5	85	Buckley et al. 1994
<i>Sebastes</i> sp1	–	245	1.5 × 0.5	0–7	Buckley et al. 1994
<i>Sebastes</i> sp2	–	59	1.5 × 0.5	9	Buckley et al. 1994

^a Fish size is expressed as total length (TL), standard length (SL) or fork length (FL)

showing no evidence of fin regeneration, allowing easy discrimination between marked and non-marked individuals. The major drawback of this method is obviously the impossibility of individual identification.

Given that implanted tags or pelvic fin excision did not affect survival, growth or body condition, would meet the requirements of mark-recapture studies, i.e. marks must not alter growth or survival (Jepsen et al. 2008). However, fish behaviour should be studied in order to evaluate possible effects in terms of vulnerability to predation and decrease in fitness in terms of resource competition.

Although *H. macraei* growth rate was extremely low (i.e. less than 1 mm in length during the whole experiment), its *K* increased up to day 100 of the experiment (in July) from ~0.46 to ~0.56. This change in body condition could be the result of the post-spawning recovery period, as the spawning period of this species ranges from December to February during the austral summer season (Barriga and Battini 2009). In addition, low water temperatures during most of the experiment probably contributed to the low growth rate measured in *H. macraei*.

Injectable fluorescent marks, such as visible implant elastomer (VIE) or injectable photonic dye (IPD), are other marking techniques often used for individual identification of small stream fishes. These methods have

been applied to estimate population size (Trajano 2001), fish dispersion (Belica and Rahel 2008; Ficke and Myrick 2009; Mitsuo et al. 2013), growth rate (Trajano and Bichuette 2007), survivor rates (Reznick and Bryant 2007) and natural selection (Weese et al. 2010). High retention rates and low mortalities have been reported applying VIE or IPD (Roberts and Angermeier 2004). However, VIA tags are less invasive than VIE or IPD because just one injection is enough to mark a fish. In contrast, when VIE or IPD are used a combination of marks is necessary to individualize fish.

The enormous population variation in spot patterning can be a useful tool for individual ID. The small percentage of individuals (less than 5 %) were misclassified owing to their homogeneous dot patterning, which is present only in a small fraction of wild populations. For example, in Pichileufu River only 3.4 % of 1334 individuals captured and released for a mark-recapture study had this pigmentation pattern (J. P. Barriga, unpubl. data). However, this small percentage can also be identified when only the largest dots in the delimited area are considered. Despite this misclassification when applying the software, these individuals may also be successfully identified by using particular characteristics such as scars or identifying specific dots on the whole fish.

Photo-identification is low-cost method, but it is time-consuming; therefore, this procedure is best suited for use in field or laboratory trials when the number of experimental individuals is low. This technique could potentially be applied to other species of the family Trichomycteridae (e.g. *Trichomycterus areolatus* Valenciennes, 1846) and to non-related species whose spot pattern is highly variable, like some Galaxiidae or Rivulidae in the Southern hemisphere and Salmonidae or Esocidae in the North.

In conclusion, selection of the identification method or combination of methods to be used must be based on clearly identified goals, the type of information needed, the degree of loss that is acceptable, and how long the study will last. None of the methods used here was a significant source of mortality or hindered fish growth. However, VIA tags had a low retention time and information was not always legible when fish were recaptured. Fin clipping was easily recognized with no evident regeneration during the study period. Pid, using spot pattern analyses as a tool for individual identification, is strongly recommended in *H. macraei*. However, as this method is time-consuming, and as a minor fraction of the population could present a homogeneous pattern of small dots, the simultaneous use of complementary methods, such as pelvic fin excision or VIA tagging is also advisable in order to facilitate identification.

Acknowledgments We thank two anonymous reviewers whose helpful comments increased the clarity of the manuscript. We thank *Dirección de Pesca Continental* of the Río Negro Province for permission to collect native fish. This study was partially funded by *Agencia Nacional de Promoción Científica y Tecnológica*, Argentina (ANPCyT, PICT 2010, No. 0262) and *Consejo Nacional de Investigaciones Científicas y Técnicas*, Argentina (CONICET, PIP No. 11220080100282). All handling, care and experimental procedures used in this research complied with the animal welfare laws stated by the Government of Argentina (Law n° 14346).

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