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Application of different external tagging methods to *Octopus vulgaris* Cuvier, 1797, with special reference to T-bar anchor tags and Petersen disks

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ABSTRACT

This paper presents a study on the persistence and effects caused by a wide variety of types of tag (loops, rabbit tags, girths, flexible plastic bands, small darts, Petersen disks, T-bar anchor tags, circular plastic tags, rectangular plastic flags, jewellery pins, commercial badges, and burning with liquid nitrogen) applied to the cephalopod *Octopus vulgaris* Cuvier, 1797, under culture conditions. Preliminary analysis of the results obtained with the different tagging systems indicates that Petersen disks and T-bar anchor tags are the most suitable types. Both provide adequate retention after 1 month (90 % and 80 %, respectively), a maximum persistence of three months, a similar cost, and they are easily recognised externally. However, Petersen disks can produce serious injuries, and require a more laborious insertion process. Therefore, in the final analysis, the insertion of T-bar anchor tags in left arm III can be considered the most appropriate system.

Keywords: *Octopus vulgaris*, tagging, T-bar anchor tags, Petersen disks.

RESUMEN

Aplicación de diferentes métodos de marcado externo a* Octopus vulgaris *Cuvier, 1797 con referencia especial a las marcas de tipo T y a los discos de Petersen

*En este trabajo se presentan datos de persistencia y efectos provocados por una gran variedad de estilos de marcas (*loops*, marcas de conejos, cinchas, cintas plásticas flexibles, lancetas, discos de Petersen, marcas de tipo T, marcas plásticas circulares, banderillas plásticas rectangulares, soportes de bisutería, pines comerciales y quemaduras con nitrógeno líquido) aplicadas al cefalópodo* Octopus vulgaris *Cuvier, 1797 sometido a condiciones de cultivo. Tras una primera revisión de los resultados obtenidos con los diferentes sistemas de marcado se concluye que los discos de Petersen y las marcas de tipo T son los más aconsejables. Ambos muestran una retención adecuada después de un mes (90 y 80 % respectivamente), una persistencia máxima de tres meses, un coste similar y ambos son detectados externamente con facilidad. Sin embargo, los discos de Petersen producen heridas considerables y requieren un proceso de inserción más laborioso, por lo que, finalmente, el sistema considerado más adecuado fue la inserción de marcas de tipo T en el brazo III izquierdo.*

Palabras clave: Octopus vulgaris*, marcado, marcas de tipo T, discos de Petersen.*

INTRODUCTION

The experiments described in this article took place within the research project titled 'Tagging and release of *Octopus vulgaris* Cuvier, 1797, paralarvae and juveniles' (CYTMAR 97-0323) and were developed at the Instituto Español de Oceanografía (IEO) in Vigo (northwest Spain). The main goal of this project was to determine the most efficient tagging systems for *O. vulgaris* paralarvae and juveniles. The first phase, on efficient internal tagging for paralarvae, has been described by the authors in a previous article (Fuentes, Iglesias and Moxica, 2000), where a chemical tagging method for statoliths with alizarin complexone was used. The present paper focuses on experiments performed using external tagging in octopus sub-adults, in order to determine the most efficient tagging technique.

There is considerable literature on finfish tagging (e.g., Nielsen, 1992; Sánchez-Lamadrid, 2001), but there are far less publications related to cephalopods, and more specifically, to the common octopus *O. vulgaris*. Especially noteworthy is the compilation made by Nagasawa, Takayagani and Takami (1993), presenting data published in Japan between 1927 and 1990 on 14 cephalopod species. Cephalopod tagging has also been indirectly addressed in other publications, such as Sakurai *et al.* (1993) and Gonçalves *et al.* (1995), who worked with *Todarodes pacificus* (Steenstrup, 1880) and *Loligo forbesi* (Steenstrup, 1856) respectively. Taki (1941), Inoue, Hamaguchi and Li (1953), Katayama and Morita (1960) showed the difficulties inherent in tagging *O. vulgaris* since, despite having used metal plaques, heat burning, wires and different colorants (methylene blue, neutral red, trypan blue, erythrosine, saffranine) on the skin or injected in the muscle, they failed to obtain satisfactory results. Domain, Jouffre and Caverivière (2000) compared *O. vulgaris* growth in tanks with marked wild individuals in Senegalese waters using Petersen disks.

In this article, a detailed description is given of the steps followed to select the most efficient tagging method, as well as the most suitable body area to tag on *O. vulgaris*.

MATERIALS AND METHODS

Capture and acclimatisation of juveniles

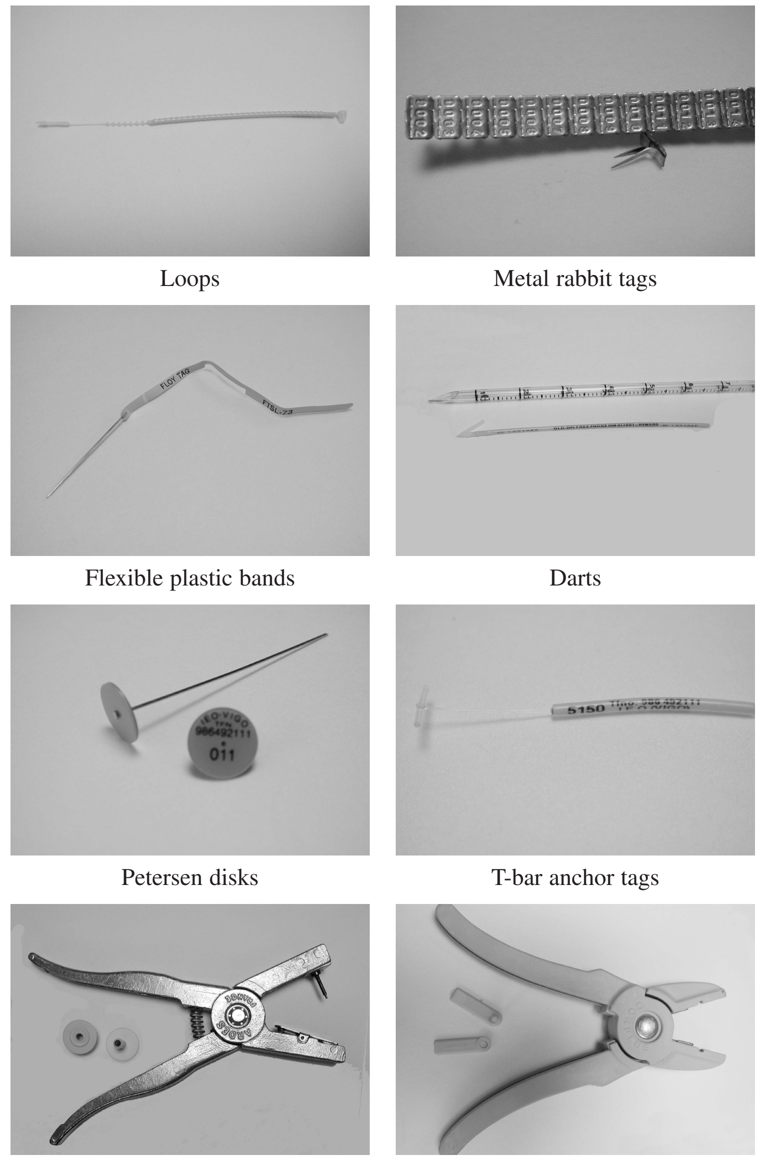
Octopus specimens weighing between 0.5 and 1 kg were caught in the Ría de Vigo between 1998 and 2001 by small local fishing boats. They were transferred to IEO facilities in 200 l tanks with properly oxygenated seawater. Before beginning the tagging experiments, the specimens were acclimatised to captivity for one week, following the recommendations on water circuit, type of tank, characteristics of den, etc., given by Iglesias *et al.* (1999). The food given consisted basically of frozen crabs *Carcinus maenas* L., 1758 although sporadically the diet was complemented with fish, mainly *Micromesistius poutassou* (Risso, 1826) and mussels *Mytilus* sp.

Types of external tag and their placement

During the first two years (1998 and 1999) a wide range of tags were tested: injected acrylic paint and cold burning with liquid nitrogen, plastic tags (loops, girths, small darts, flexible plastic bands, badges, earrings or jewellery pins, rabbit tags and Petersen disks) and those inserted with a gun (T-type anchor tags or Corplus® circular and rectangular ones). Following is a brief description of each one (some of them are showed in figure 1).

- Loops (Hallprint®, 'Self-locking loop tags' series SLA). The ends of this 5" (118.5 mm) plastic cord join in a mechanism which prevents tag loss. These were manually placed in the mantle and arm.
- Metal rabbit tags (Hauptner®). A rectangular numbered plaque (10 mm by 5 mm) with pointed prongs on the base which pierce the structure to be tagged –in our case, the mantle– and once inside are bent into place.
- Small commercial plastic girths, length 100 mm which were fixed round the arms and in the external end of the mantle.
- Flexible plastic bands (Hallprint® 'Polyethylene streamer tags', series PST), especially designed for crustaceans (shrimps, crabs, small lobster, etc.). The band pierces the structure to be tagged (mantle) drawn by a needle, which is later withdrawn. The two ends of the bands are free whilst the middle part goes through the structure to be tagged.
- Darts (Hallprint®, 'Small plastic tipped dart tags' series PD). They have a hook-like point and are inserted by being placed into a thin tube with a sharp end, which is introduced into the area to be tagged (arms) and then slow-

Figure 1. Different kinds of tags used in the present study



Loops — Metal rabbit tags — Flexible plastic bands — Darts — Petersen disks — T-bar anchor tags — Circular plastic tags — Rectangular plastic tags

ly withdrawn, making sure that the point of the dart is anchored.

- Petersen disks (Floy Tag & MGF. CO., INC.). The 9/16" (13.3 mm) diameter disks with a central hole are made of vinyl protected against UV; the nickel pins measure 3" (11.1 mm). The system consists of two disks joined by a pin which can pierce any part of the body. The inscription on this type of tag is on the surface of one of the disks and their insertion, unlike the T-bar anchor tags is, completely manual.
- T-bar anchor tags (Hallprint® 'T-bar anchor tags', series TBF-2 fine) are pieces of flexible T-shaped plastic. The short bar of the T (8 mm) is inserted into the animal's body and the longer bar (35 mm) bears an inscription, which includes the tag number, address and telephone. This tag is inserted using a tagging gun designed by the same company.
- Circular plastic tags (Insvet Corplus®). A system similar to Petersen disks; that is, two plaques of approximately 20 mm diameter, joined by an element which pierces the body structure (mantle), but in this case, the pin is replaced by a larger diameter element. These tags are inserted with a specific applicator.
- Rectangular plastic tags (Corplus®), a tagging system similar to the previous one, but with

the difference that the plaques are rectangular (approx. 30 mm × 10 mm). Their insertion also requires an applicator from the same firm.

- Nitrogen burns were produced by pressing the animal's skin with rods previously submerged in liquid nitrogen. The numbers on the end of the rods are then tattooed on the octopus mantle.
- Others. Other systems were tested such as, earring bases, commercial badges on the edge of the mantle, and even subcutaneous injection with acrylic paint in the arms and mantle.

Most of these (loops, rabbit tags, flexible plastic girths, darts, Petersen disks and T-bar anchor tags) carry an inscription which makes individual tracking possible.

To select the most appropriate tagging place, insertions were made in the mantle and the arms. An experiment was also carried out to determine which arm was the most suitable for use with this type of tags, so tests were made in triplicate on left arms I, II, III and IV.

Preliminary experiments

Throughout 1998 and 1999, numerous tests were carried out, which made it possible to evaluate the degree of retention rate in a wide range of external tags on the species *O. vulgaris*. All these experiments took place in 1 m^3 tanks in an open water circuit using 10 specimens per test, and had a duration of 1 month. The tags were those cited in the previous methodology section. In each experiment, calculations were made regarding degree of retention, injuries produced, and maximum persistence, so that at the end of this process, it was possible to select the most suitable tag.

Comparison between T-bar anchor tags and Petersen disks

From the preliminary experiments, the conclusion reached was that the most persistent tags were the T-bar anchor tag and the Petersen disks. Consequently, in 2000-2001, more exhaustive tests were carried out, with the aim of determining the efficiency of each one and finding out which was the most effective placement.

Before comparing Petersen disks and T-bar anchor tags, another experiment had been designed to select the most suitable arm for tagging, in which 12 octopi were tagged, three replicates each for the I, II, III and IV left arms with Petersen disks in individual 100 l tanks. Right arms were not tested so as not to interfere so as to not interfere with the male reproductive function (as is known, the third right arm, or hectocotylus, is used by males to copulate). This experiment lasted 1 month, and the number of injuries and lost tags were monitored weekly.

To compare Petersen disks and T-bar, the following methodology was used.

To avoid the octopi becoming hyperactive during the tagging process, they were anaesthetised by immersion for 5 minutes in cold seawater (4-5 ºC), to which a few drops of ethyl alcohol had been added.

The tests were carried out with 2 replicates for each type of mark, both in the mantle and in left arm III. The experiments had a duration of 1 month and were kept in 2 000 l capacity tanks with 10 specimens in each, so a total of 80 specimens were tagged. Weekly, for the period of 1 month, a control was carried out, recording the weight of specimens in each experiment, the number of lost tags and any other kind of observation related to tag evolution (correct fixing, appearance of relevant injuries, etc.). Finally the possible effect of the marking procedure on growth was analysed in both types of tags.

RESULTS

Preliminary experiments

Concerning the different types of marks utilised, it was observed from the first-year results that most of the marks were lost in the first two weeks of the experiments, confirming the difficulty of tagging cephalopods. A brief summary of these results obtained for each system are presented in figure 2. None of the loops lasted more than 9 days. The circular plastic tags (Insvet Corplus®) lasted between 4 and 14 days; only one of them reached 27 days. Only one of the metal rabbit tags remained 6 days joined to the octopus. The octopi freed themselves from the girths very easily, and when this was not

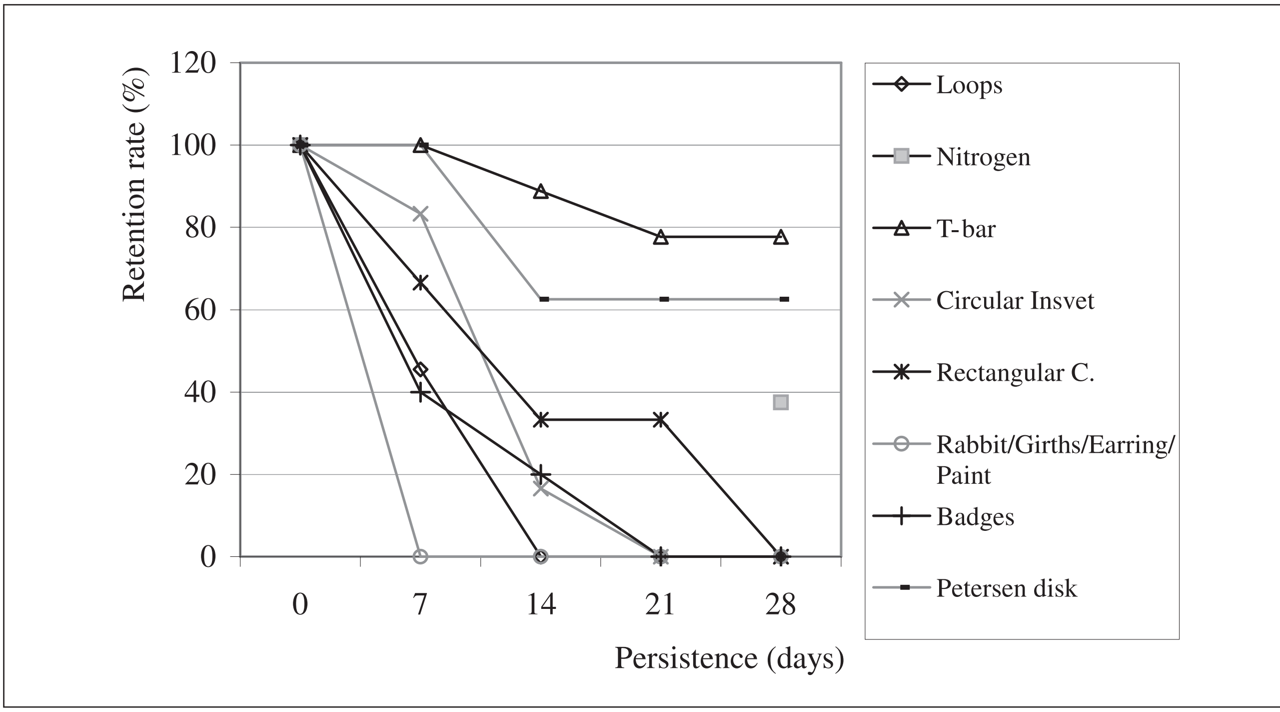


Figure 2. Retention rate (%) for each type of mark from preliminary experiments (1 month long)

the case, they caused considerable injuries where they had been placed. The majority of the badges became unpinned on the same day that they were inserted; one lasted 10 days. The jewellery bases did not survive long, except in one specific case, which lasted 42 days. The paint disappeared almost immediately, leaving no trace. After a month, only 37 % liquid nitrogen burns were visible, but their numbers were unreadable; the lack of firmness and scant consistency of octopus muscle, coupled with its capacity for regeneration, make tag retention difficult.

On the other hand, the results of those experiments carried out with T-bar (anchor) tags were most satisfactory, recording a retention rate of 77 % after a month (figure 2), and a maximum persistence time of 89 days (data recorded after the 1-month experiment). In the case of the Petersen disks, a similar retention rate (62 %) was obtained after a month, compared with the T-bar tags, but wounds soon appeared in the insertion area when Petersen disks were used.

From these results, it was concluded that among all the different types of tags used, the most suitable were Petersen disks and T-bar anchor tags. The study was therefore oriented towards a more detailed comparison between these two types.

Comparative study between Petersen disks and T-bar anchor tags

Evaluation of the most suitable arm

From the 12 individuals marked in left arms I, II, III and IV, only one specimen, specifically one tagged on left arm II, lost its tag before a month had elapsed (figure 3). As the results showed no preference among treatments and retention percentages were similar, left arm III was chosen for future experiments.

Marking effect on growth

Figure 4 shows the mean wet weight observed in the 1-month period of growth for the individuals marked with T-bar and Petersen disk tags. Initial mean weights were 846.5 g for T-bar and 843.6 g for the Petersen disk, reaching final weights of 1 157.9 g and 1 000.3 g, respectively. Growth in weight was adjusted to the following exponential equations:

$$\text{T-bar: } y = 865.49\ e^{0.0384x};\ R^2 = 0.8897$$
$$\text{Petersen disk: } y = 849.35\ e^{0.0773x};\ R^2 = 0.9867$$

No significant difference in growth was found between the two groups ($\alpha < 0.05$), indicating that

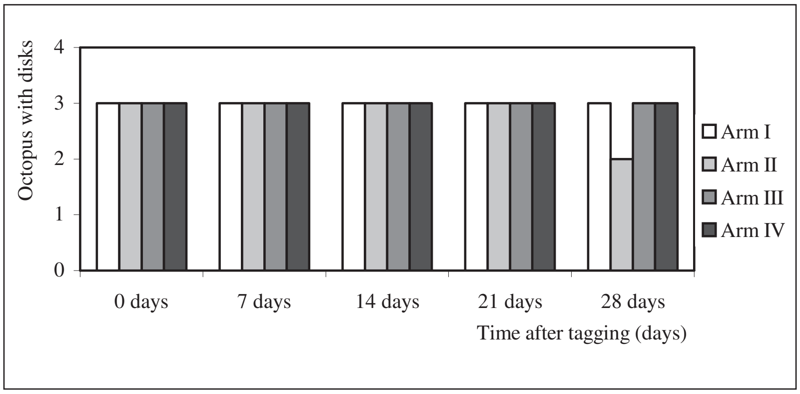


Figure 3. Persistence (days) of the tags placed in different arms (right arms I, II, III and IV)

there was no negative effect of the marking procedure on growth.

Persistence obtained in both types of tags

In figure 5, it can be seen that inserting the tag in the arm was more effective than in the mantle, both with Petersen disks and T-bar anchor tags. Of those markers inserted in the mantle, only one of the 10 disks reached day 28 of the experiment, and all the T-tags were lost during the first week.

Regarding the comparison between the disks and the T-tags using left arm III as a site, average values of 80 % T-tags and 90 % disks remained in place for the 4 weeks of the test (figure 5). Both marks presented a maximum persistence of more than 3 months (data recorded after the 1-month experiment). However, it is necessary to point out that disks caused cuts and other injuries in the insertion area after the first week of the experiment.

DISCUSSION

In this work the great difficulty in tagging cephalopods when compared with fish tagging has been confirmed. Considerable differences exist between the viability of same tagging technique being used with fish and cephalopods. *O. vulgaris* muscle is less firm than that of fish, and therefore tag anchoring is less safe and reliable; nor do cephalopods have no hard structures, such as a backbone, on which to thread the tags. At the same time, the great agility inherent to octopi, along with the whole range of movements they can make with their arms, play a decisive part against any tagging system, since these characteristics enable them to remove their own tags or those of others. All of these factors have complicated considerably the choice of a suitable tag for these animals.

The retention rate and effects on the tagged specimen with regard to survival, injuries and growth, along with difficulties related to application processes and tag detection, are fundamental

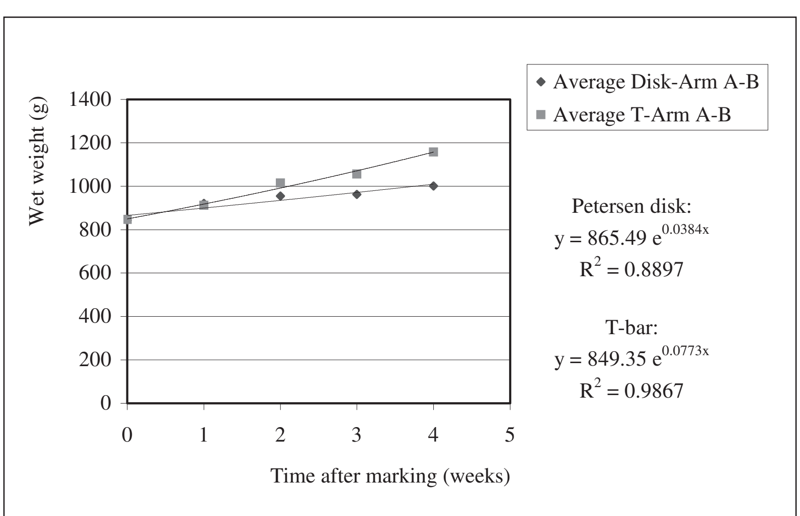


Figure 4. Growth in mean wet weight (g) from individuals marked with Petersen disks and T-bar anchor tags during 1 month

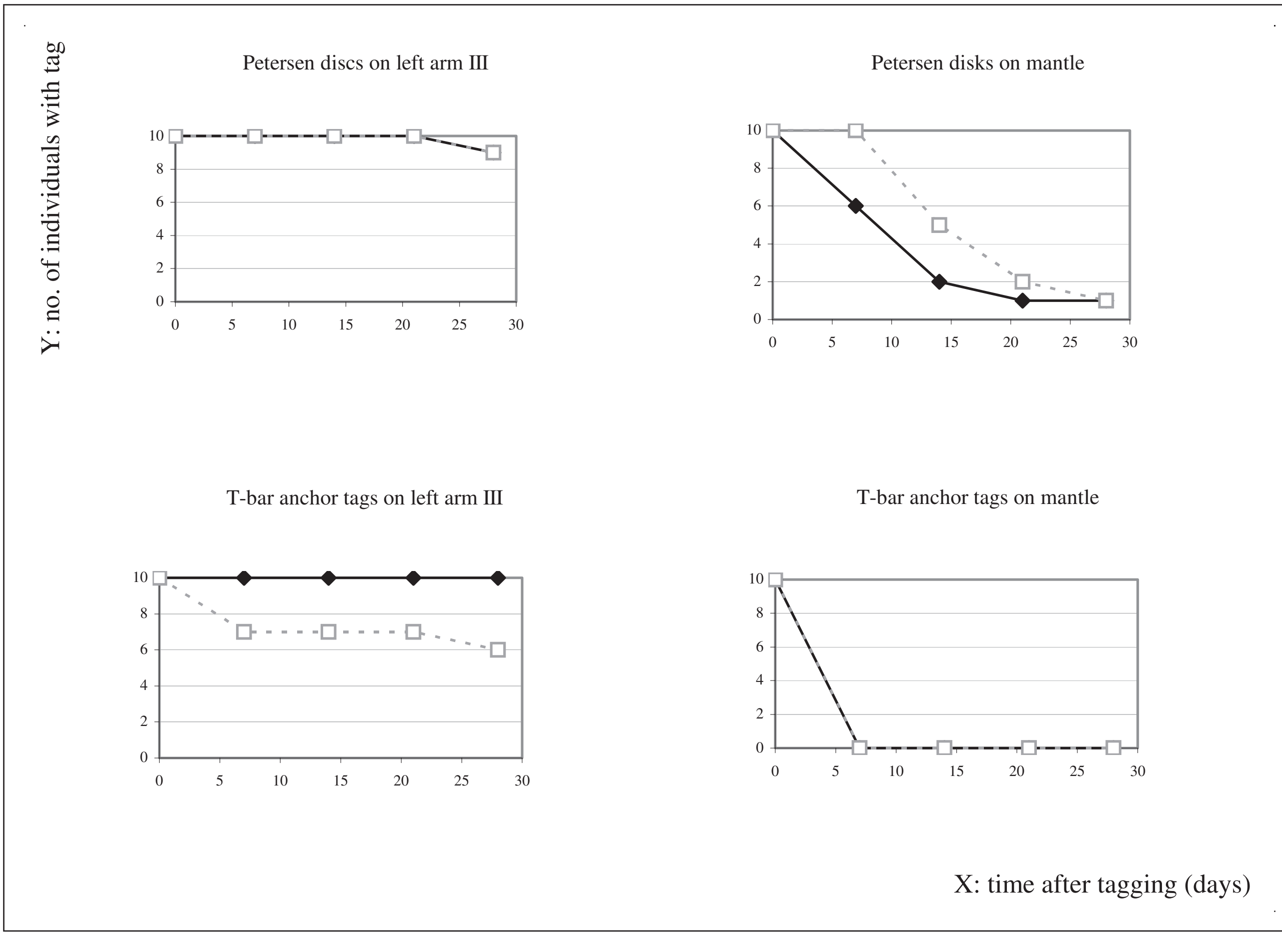


Figure 5. Persistence obtained in a comparative study between Petersen disks and T-bar anchor tags. Replica 1: —◆—; replica 2: -□-

issues to consider when a tagging system is to be chosen for enhancement programmes (Collins, Smith and Heyward, 1994). Each of these aspects was analysed for the tags used in this experiment. Regarding retention rate from preliminary experiments, we had to reject such systems as acrylic paint injections, plastic girths, metal rabbit tags, commercial badges, liquid nitrogen burns, and loops. The maximum persistence for earring bases, circular plastic tags (Insvet) and rectangular flags (Corplus®) was approximately 30 days, but only a very low percentage of these actually lasted that long. In addition, the last two types of tags mentioned caused considerable injuries. Subsequently, only the Petersen disks and the T-bar anchor tags passed this first screening.

Several authors have already dealt with the subject of tagging *O. vulgaris*. Most of these works are by Japanese authors and almost none are recent. Taki (1941) compared the permanence of several types of tags and marks to identify *O. vulgaris* individuals kept in aquariums. The tags evaluated were pieces of sewn material, metal plaques, removal of a sucker, and dyeing techniques using trypan blue on the dorsal part of the mantle. He observed that the first two systems were easily removed by the animals; the excision of a sucker was only valid during the two months of regeneration, and that trypan blue produced stains which were visible up to six months after application. Inoue, Hamaguchi and Li (1953) maintained that burning is effective for *O. vulgaris*, and attained a 90-day persistence. Regarding this particular tagging system, Katayama and Morita (1960) suggested that the best place for its application is the posterior end of the mantle, because there it is easily and does not change its shape. However, we observed poor retention rates for cold burning. Itami (1964) also showed that 35.7 % of the animals tagged in this way died from the effects, but these were particularly small specimens.

Takeda *et al.* (1981) again used trypan blue and detected marks a month after tagging. These same authors showed that wires used to pierce the mantle or the umbrella were pulled out by the octopi, in most cases in less than 20 days. This fact is agreement with the results of this paper, which clearly stated that better retention rates were obtained when octopi were marked in the arm rather than in the mantle.

Studies carried out more recently by Tsuchiya, Ikeda and Shimizu (1986) examined the effectiveness of a wide range of tags (anchor tag, dart tag, Petersen disks, fingerling tag, metal ring and nylon thread) and dyes (methylene blue, neutral red, erythrosine and saffranine T). They showed that in general, tags (external marks) were not effective, some of them being removed by the arms and others becoming loosened by the injuries caused by the tagging process. On the other hand, the methylene blue and neutral red intramuscular injections under skin of the mantle to form 1-2 cm diameter stains, were successful insofar as persistence was concerned, and hardly affected the tagged animals. This method of individual recognition using dyes could be viable at an experimental level, but it does not permit individual identification of tagged specimens when the intention is to use a tag recognisable to fisherman in an enhancement programme.

In this work, Petersen disks and T-bar anchor tag were the only methods that passed the first screening and provided adequate retention after 1 month (90 % and 80 %, respectively). Both had a maximum persistence of three months and a similar cost; moreover, they are easily recognised externally. Domain, Jouffre and Caverivière (2000) also used Petersen disks in growth studies, and they obtained promising persistence values (up to 177 days). It must be remembered, however, that the disks require a laborious manual insertion process, and that the cuts and other injuries produced can be very serious and increase mortality.

Consequently, we recommended the use of a T-bar anchor tag inserted in left arm III to carry out short-term studies (1-3 months) on the biology and distribution of *O. vulgaris* juveniles in their natural environment.

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