Fisheries Research 187 (2017) 209–217

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Full length article

Comprehensive evaluation of passive tags show no adverse effects in an economically important crustacean

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ARTICLE INFO

Article history:
Received 12 February 2016
Received in revised form
28 November 2016
Accepted 29 November 2016
Handled by George A. Rose
Available online 7 December 2016

Keywords:
Tagging
Nephrops
Crustacea
Mortality
Growth

ABSTRACT

Animal tagging can reveal important information about population size, growth and survivorship, however the accuracy of this information relies on establishing that tags are retained and that there are no adverse tag-associated effects. A suite of potential effects associated with Coded Wire Tags (CWTs) was investigated in captive and in wild *Nephrops norvegicus*. The study included a total of 232 *N. norvegicus* (107 tagged and 125 untagged) which were held captive in the laboratory over 12 months. No tag loss was recorded in these captive *N. norvegicus* despite moulting having occurred in 77/107 tagged individuals. Recovery of tags from the initial tagging site or otherwise was compared in captive and wild animals by tagging and releasing an additional 205 *N. norvegicus* into Clew Bay, Co. Mayo in Ireland, and recapturing them approximately one year later. Tag movement away for the site of injection consistently occurred in ~23% of cases (all treatments combined). In ~10% of individuals, tags were found at the individual's extremities and, due to their precarious position, these tags would certainly have been lost during the next moult. However, tag movement was very consistent across groups organized by sex (male/female), mobility (captive/wild) and growth increment (high/low), therefore the proportion of tags lost during longer experiments is not predicted to be biased across these groupings. Mortality was not increased in tagged individuals relative to controls in large (above median) or small (below median) size classes in either males or females. In fact, mortality was *reduced* in tagged females of both sizes compared with the untagged group. An apparently positive effect of tags was that the frequency of moulting was higher in tagged males and females compared with their untagged counterparts. Differences in growth increment at moult were also seen in males, with tagged individuals growing up to 50% more. While the latter was conceivably a sampling artifact, the beneficial effect of tags on moulting warrants further study. Overall, the results indicate no negative effects of CWTs on *N. norvegicus* growth and survival.

1. Introduction

Passive tagging is a vital tool in population studies, providing information on population size (Lincoln, 1930; Petersen, 1896), residency (Abecasis et al., 2009; Neat et al., 2006), migration routes and patterns (Castro-Santos et al., 1996; Roni et al., 2012), minimum survivorship (Pistorius et al., 1999) and growth (Pratt and Casey, 1983). But tagging animals which moult during growth is difficult because tags that are placed externally will generally be lost over time with the exoskeleton (Cooper, 1970). Moulting can occur every few weeks in the fastest-growing phases (Conan, 1978) and this can place individuals in a precarious position since animals are vulnerable while the new exoskeleton develops and hardens. For the purposes of scientific experiments, it is imperative that a tagging technique does not interfere with the moulting process, or make tagged animals more vulnerable to mortality during either moult or intermoult periods (Thorsteinsson, 2002). In addition to addressing mortality concerns, tags should not be associated with increased morbidity including effects on the daily range of movement and feeding, which could affect growth rates (Weingartner, 1982). For this reason, tags should be unobtrusive and generally small enough relative to the body size of the study species. In addition, there are practical concerns relating to the ability to detect

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http://dx.doi.org/10.1016/j.fishres.2016.11.025
0165-7836/

tags. For example, external tags containing contact details can help to maximise returns, whereas this is not possible with internal tags.

Internal tags are often the preferred method in studies involving Crustacea, as these overcome the constraints associated with moulting (Håstein et al., 2001; Thorsteinsson, 2002). Previous studies have employed a range of internal tags including Passive Integrated Transponder (PIT) (Black et al., 2010; Bubb et al., 2002), Visible Internal Elastomer (VIE) (Davis et al., 2004; Linnane and Mercer, 1998) and Coded Wire Tags (CWTs) (Davis et al., 2004; Linnane and Mercer, 1998; Okamoto, 1999; Sharp et al., 2000). Accurate growth estimates from tagging are particularly valuable in crustacean fisheries because verified aging techniques are lacking in most species. This presents severe challenges to sustainable management of commercially valuable crustacean fisheries such as *Nephrops norvegicus*, also known as Dublin Bay prawn or Norway lobster. There has been a dramatic increase in the global landings of this species to a peak of 76,000 t in 2007 (FAO FishStat, 2015), and this is one of the most commercially important species in EU fisheries (Lauria et al., 2015; Ungfors et al., 2013). Although growth rates in this species are usually calculated using indirect methods including length-cohort analysis (Ayza et al., 2011; Bailey and Chapman, 1983; González-Gurriarán et al., 1998; Hillis, 1979), such approaches may be unsuitable because of the widespread use of fisheries-dependent data with inherent homogenous size structure, poor capture rates of juveniles and other uncertainties (Haynes et al., 2016 and references cited therein). Previous tagging studies in *N. norvegicus* have returned less than 6% of the originally tagged population (Chapman, 1982; Hillis, 1985; Ulmestrand and Eggert, 2001) and these studies raised the possibility that adverse effects of the tags may have contributed to the poor rate of returns. This question remains open since no studies have examined any effects associated with internal tags in *N. norvegicus*.

The present study evaluated Coded Wire Tags (CWTs) which were injected into *N. norvegicus* over a range of sizes to measure tag-associated effects on i) mortality and ii) morbidity, including frequency of moulting and increment at moult. During the recovery of tags, it became apparent that a proportion of tags moved from the initial tagging site. This movement is of interest as it could conceivably increase chances of mortality or affect tag retention. Therefore a specific analysis was carried out to determine the proportion of tags that moved for different groups, broken down by sex (male/female), mobility level (whether tagged individuals were captive in aquaria or whether they were growing in the wild) and growth increment (whether the animals which grew by larger increments had a higher probability of tag movement).

2. Materials and methods

2.1. Tagging with coded wire tags

N. norvegicus were tagged with a sequential Coded Wire Tag (CWT; Northwest Marine Technology Inc.). This is a passive tag made of stainless steel, with a unique identifying code imprinted on every tag. The smallest available CWT is 1.1 mm length, 0.25 mm in diameter, and this size was chosen for the present study. Although other studies have tagged in the opening of the branchial chamber, or in appendages such as legs (Bailey and Dufour, 1987; Krouse and Nutting, 1990), the abdominal musculature was chosen as a tagging site in the present study to prevent potential tag loss. Tagging inside the musculature was preferred in order to reduce the possibility of a tag moving into the circulatory system and to prevent potential obstruction or infection (McMahon and Burnett, 1990). Although tagging in an appendage would prevent such negative-tag-associated effects, appendages such as legs and chelipeds are frequently injured, lost or autotomized, especially during handling or fishing operations so tagging in the musculature was considered a more suitable location. Moreover, due to small size and biologically inert materials, CWTs present no hazard to humans if ingested (NASCO, 2005).

Tags were loaded, one at a time, into a syringe for injection into the ventral musculature of the 4th abdominal segment (numbering segments from the posterior of the animal). Tagging ventrally allowed a better grasp of the animal during the injection process and, as the ventral exoskeleton was slightly thinner than the dorsal surface, injecting through this side was also easier. Dissections were carried out after 24 h in a small subsample of tagged individuals to establish that tags had not moved position over this period and also to satisfy the investigators that no obvious injuries were evident around the puncture wound (Fig. 1).

2.2. Tag retention & tag-associated mortality

Tag-associated mortality was evaluated by holding tagged and untagged, i.e. 'control', *N. norvegicus* in the laboratory, specifically in a flow-through aquaculture system at NUIG Carna Laboratories. Individuals were randomly assigned to tagged ($n_{females} = 63$; $n_{males} = 44$) and untagged ($n_{females} = 58$; $n_{males} = 67$) groups, ranging in size from 26.2-53.0 mm carapace length (CL) in males, and 23.5-41.2 mm CL in females. There were slight *a priori* size differences in both groups of males ($mean_{untagged} = 36.6$ mm and $mean_{tagged} = 34.8$ mm) and females ($mean_{untagged} = 34.7$ mm and $mean_{tagged} = 33.7$ mm), but these were not statistically different (males, $t = 1.78$, df = 107, $p = 0.076$; females, $t = 1.77$, df = 119, $p = 0.078$). All captive *N. norvegicus* were maintained in individual polyethylene tubes which were vertically stacked and attached by cable tie to a plastic mesh inside a 3 m diameter water tank. The size of tubes was customized to the individual size and large enough to not prevent individuals from moulting. Animals were fed on weekly rations of frozen blue mussel meat (*Mytilus* spp., −approximately one mussel per week per individual) and held at ambient water temperatures throughout the experiment (mean ± SE, 11.5 ± 0.13 °C, maximum = 18.9 °C (July 2013), minimum = 5.9 °C (February 2014)). Captive individuals entered the experiment a few weeks apart as these were fished and delivered from local fishermen in 'batches', but for statistical comparison, the period for which mortality was evaluated was a consistent nine month period for all individuals, even though the specific nine month period differed slightly between individuals (~July 2013 to March 2014). Checks for mortality were made during feeding or growth measurement tasks (see below). Checks for tag retention were made approximately bi-monthly by 'scanning' which involved passing tagged individuals over a metal 'T-bar' detector designed specifically for CWTs.

In addition to examining tag-associated effects in captivity, we examined tag recovery in a wild population. 1177 *N. norvegicus* were captured in Clew Bay Co. Mayo (53.840626N, −9.695630W) using baited creels (='pots'). These individuals were captured in several batches between April and June in 2013. All individuals were tagged, as described above, and allowed to recover in the Carna holding system before releasing them back to Clew Bay several weeks later. 207 *N. norvegicus* carrying tags were re-captured during Summer 2014, after which time they had been at liberty for 344 days, on average. These were taken to the laboratory and identified by dissecting CWTs out of the abdomen and other body areas (see 2.4 below). In two individuals, tags were lost, so the overall sample size for the wild tagged experiment was n = 205. Wild-tagged males and females were similar sizes ($mean_{males} = 34.3$ mm (26.9–40.2 mm), $mean_{females} = 33.3$ mm (22.0–44.6 mm)) −Please see Haynes et al. (2016) for more details about the wild tagging experiment.