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EXECUTIVE SUMMARY

McKenzie, J.R.; Diggles, B.; Tubbs, L.; Poortenaar, C.; Parkinson, D.; Webster, K.; Miller, N. (2006). An evaluation of a new type of plastic coated PIT tag for tagging snapper (*Pagrus auratus*).

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Two sizes of Plastic Infusion Process (PIP) coated passive integrated transponder (PIT) tags were developed for use in the 2002 SNA 8 tagging programme (12 mm and 23 mm PIP). On the basis of live testing there was some evidence that the 23 mm tag may induce marginally higher mortality in snapper than the 12 mm tag, but in tests the two tags produced similar results. The overall result from live testing was that there was no evidence that injection with a PIP tag produces any significant long-term deleterious effect on mature snapper.

Mortality levels associated with trawl capture PIP tagging and release were equivalent to levels observed for trawl capture and tagging with coded wire or dart tags.

The 12 mm PIP was found to be robust against prolonged pressure exposure down to 40 atmospheres and chemical leaching or intrusion.

The observed lack of failure of any of the 132 double tag pairs of 12 mm PIPs recovered from released snapper indicates that their expected reliability factor for periods up to two years is better than 99%.

1 INTRODUCTION

The identification and detection features offered by Passive Integrated Transponder (PIT) tags make them superior to the coded wire tags (CWT) and external plastic dart tags used in previous snapper tagging programmes. PIT tags were the tag of choice for the 2002 SNA 8 tagging programme.

When the SNA 8 tagging programme was first mooted in 1999, all commercial brands of PIT tags suitable for fish use were glass encapsulated. Plastic coatings for the PIT electronics had been trialled during the early 1980s when tag technologies were developing (Dean Park, president Biomark Ltd, pers. comm.); however, none of the tested polymers were found to perform as well as glass. Glass is an ideal encapsulation medium for PIT tags in that it is biologically inert; moulded as a capsule it can protect against high pressures; it has almost zero permeability to gasses and water; it is low cost and easily moulded. PIT tag manufacturers saw no real commercial incentive to develop alternative coating mediums to glass.

Initially, the NZ fishing industry had supported the use of glass PIT tags in snapper research. However, after taking food-safety advice, industry withdrew support for the 2002 SNA 8 tagging programme claiming that the risk of glass contamination of product was unacceptably high. The basis of their concern was that snapper are sold and exported largely unprocessed and consequently there is a small but real possibility that a glass tag would find its way onto a dinner plate.

After a series of meetings between NIWA, the Ministry, and fishing industry representatives, the industry consented to the use of PIT technologies on the 2002 SNA 8 tagging programme under the proviso that tags were coated in food grade plastic. NIWA was contracted to develop an alternative plastic coating process and demonstrate its viability.

The technical details of the coating development and final manufacturing process are not covered in this report. In summary, the final coating involves imbedding and infusing the PIT electronics in food grade epoxy resin so that no significant air spaces remain in the medium, either in the surrounding epoxy or in recesses within the tag electronics. The tag electronics and the epoxy coating are housed within an acrylic outer shell (Figure 1), which acts as a mould during coating and later gives the tag additional rigidity to withstand the forces of mechanical injection into a fish. The plastic coated tag NIWA developed is called a Plastic Infusion Process tag or PIP tag.

There are two main PIT technologies for transmitting multiple-bit binary information: half duplex (HDX) and full duplex (FDX) (Finkenzeller 2003). HDX tags have a higher read range (detectability) but there are limitations on size, the smallest variants being 20 mm in length. In contrast, the FDX tags can be manufactured down to 8 mm in length. Two sizes of PIP tag were developed and trialled; a 23 mm PIP comprising Tiris RI-TRP-RRHP 134.2 kHtz HDX electronics and a 12 mm PIP comprising Destron Fearing TX 1400L 134.2 kHtz FDX electronics (Figure 2).

The various sections in this report cover a series of experimental evaluations undertaken on the NIWA PIP tag to determine its suitability for use in the 2002 SNA 8 tagging programme. Each section is largely experimentally discrete; the overall conclusions from the work are summarised in the final section. The evaluations focus on three aspects: comparisons of initial mortality in snapper tagged with PIP, CWT, and dart tags; an evaluation of stress, histological response, and heavy metal uptake in live snapper three months after PIP tagging; and the durability of PIP tags under exposure to extreme pressure and chemical environments.

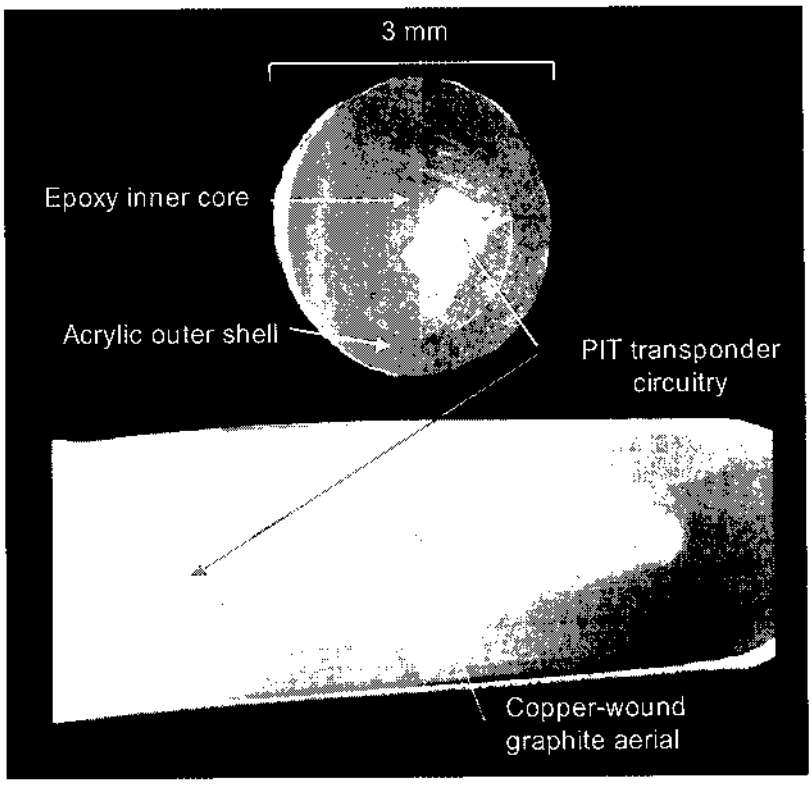


Figure 1: **Scanning electron microscope images of longitudinal and transverse sections through the 12 mm PIP tag.**

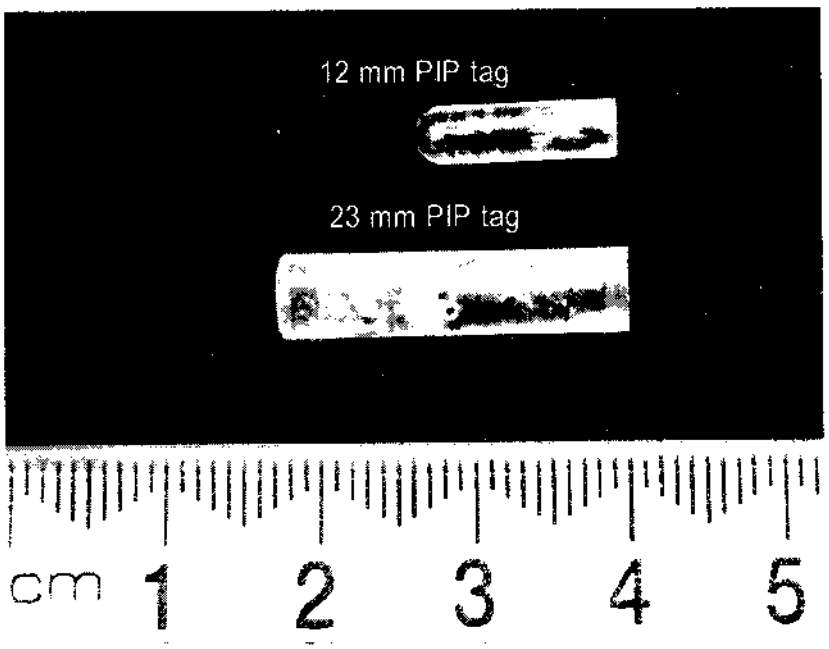


Figure 2: **12 mm (Destron Fearing TX 1400L FDX) and 23 mm (Tiris RI-TRP-RRHP HDX) PIP tags.**

2 PIP TAG PLACEMENT MORTALITY

2.1 Introduction

Past snapper tagging studies have used external dart tags (Davies et al 1999) and internal coded wire tags (McKenzie & Davies 1996) as markers. Tag and release mortality associated with trawl and longline capture methods have been estimated experimentally for both types of tag (McKenzie & Davies 1996). Longline mortality estimates range between 5 and 30% and are largely influenced by capture depth. Trawl mortality estimates range between 20 and 50% and are dependent upon shot weight and fish length (McKenzie & Davies 1996). Mortality rates associated with the 23 and 12 mm PIP tags were unknown.

A scaled-down repeat of mortality studies conducted in 1992 (dart tag) and 1994 (coded wire tag) was conducted to investigate the hypothesis that: initial mortality in PIP tagged snapper trawled from depths of 20 to 30 m is not "significantly greater" than that observed for CWT or dart-tagged snapper captured under the same conditions.

2.2 Methods

A 20 m commercial fishing trawler equipped with a high-opening bottom trawl net, undertook eight 20-minute tows in depths between 20 and 30 m. Snapper deemed fit for tagging were injected with either 23 mm or 12 mm tags on alternate tows (4 tows per tag-type). Handling procedures were consistent with those used in previous studies (McKenzie & Davies 1996). A PIP tag was injected into the peritoneal cavity of each fish with an appropriate gauge hypodermic syringe. After tagging, fish were retained on board the vessel in a large tank and carefully transported to a circular sea cage (20 m diameter x 5 m deep) into which they were released.

The assumption is made that no additional mortality was induced by transportation and/or the holding net per se. To test the assumption, 100 untagged pristine and minimally handled snapper were released into the holding cage before tagging began. These 'control' fish were captured by longline from depths less than 12 m.

Fish were monitored in the sea-cage for 19 days, the net being cleared of dead fish, twice daily, by divers. At the end of 19 days, all remaining live fish were removed from the net.

All fish surviving to the end of the study were classified according to five condition factors using the following subjective scale:

Condition factor	*Condition classification*
R/L EYE:	**N**ormal, **C**loudy, **B**lind
Scale Rot:	**N**one, **S**light, **M**arked(< 20%), **E**xtensive
Fin Rot:	**N**one, **S**light, **M**arked, **E**xtensive
Gut Cavity:	**N**ormal, Obvious infection
Tag Wound:	**N**ot visible, **V**isible, Obvious infection

Fish receiving two or more 'extreme' scores were classified as dead; it was assumed that these fish would have eventually died of their injuries.

A randomisation procedure (bootstrap sampling without replacement) was used to compare mortality observations from the PIP tag study with observations from combined CWT and Dart tag studies. The bootstrap procedure generated the probability of getting a worse mortality in PIP tagged snapper by chance. Bootstrap probabilities of 5% or less were deemed to be significant.

The number of control fish dying in each experiment was assumed to provide a measure of mortality attributable to the holding net, for example, mortality amongst control fish in the dart/CWT studies was 2% (4 mortalities amongst 196 controls). To account for possible experimental differences in control fish mortality, dead snapper in the dart/CWT and PIP experiments could come alive again in each bootstrap with a probability equivalent to the observed level of control fish mortality, e.g. 2% for the dart/CWT tagged fish.

The number of trawl tows was limited to eight and it was not expected to capture more than 150 fish per tag type. Based on this expectation, 'length' was stratified a priori into two categories: 25–29 cm and over 30 cm. 'Shot-weight' bin-classes were defined a priori as: 0–75kg for the first bin incrementing by 50kg intervals thereafter.

2.3 Results

After the completion of eight trawls, 234 snapper had been tagged; 84 with 23 mm tags and 150 with 12 mm tags (Appendix 1). An additional 77 snapper were captured as controls. These fish were assigned to length and shot-weight bin-classes as shown in Table 1. There were 498 observations corresponding to these classes from the previous mortality studies.

Table 1: Number of experimental observations per shot weight and length classes.

		Length Class	
Tag type	Shot weight kg	25-30	≥30
12 mm	0–74	40	9
	75–124	41	9
	425–474	30	21
23 mm	0–74	31	0
	75–124	23	30
	425–474	0	0
dart/CWT	0–74	40	120
	75–124	80	193
	425–474	11	54

The null hypothesis that PIP tag initial mortality was higher than dart/CWT tag mortality could not be rejected in five out of six comparable treatment classes for 12 mm PIP tag initial mortality (Table 2). The same null hypothesis could not be rejected in one out of three comparable treatment classes for the 23 mm PIP tag (Table 3).

Table 2: Bootstrap adjusted mortalities for 12 mm PIP and dart/CWT tagged snapper by treatment class; test of hypothesis that PIP tag initial mortality was higher than dart/CWT tag mortality.

		Adjusted (%) mortality			
Length (cm)	Shot	12 mm	cwt/dart	P[CWT<PIT]	Significant
25–30	0–74	29.58	36.66	0.6609	
	75–124	28.71	42.78	0.8955	
	425–474	66.25	62.17	0.2754	
over 30	0–74	20.23	15.49	0.2009	
	75–124	30.33	19.78	0.1337	
	425–474	64.85	21.79	0.0004	***

Table 3: Bootstrap adjusted mortalities for 23 mm PIP and dart/CWT tagged snapper by treatment class; test of hypothesis that PIP tag initial mortality was higher that dart/CWT tag mortality.

		Adjusted (%) mortality			
Length (cm)	Shot	23 mm	cwt/dart	P[CWT<PIT]	Significant
25–30	0–74	58.42	36.62	0.0264	***
	75–124	39.42	42.75	0.5121	
	425–474	–	–	–	
over 30	0–74	–	–	–	
	75–124	54.20	19.77	0.0001	***
	425–474	–	–	–	

A direct comparison of mortality between 12 and 23 mm PIP tagged snapper showed 23 mm tagged snapper had significantly worse mortality in two out of three comparable treatment classes (Table 4).

Table 4: Bootstrap mortalities for 23 mm PIP and 12 mm PIP tagged snapper by treatment class; test of hypothesis that a higher 12 mm PIP tag mortality than would be observed by chance.

		(%) Mortality			
Length cm	Shot	23 mm	12 mm	P[12mm<23mm]	Significant
25–30	0–74	64.39	32.44	0.0016	***
	75–124	43.39	31.64	0.1125	
	425–474	–	–	–	
over 30	0–74	–	–	–	
	75–124	59.88	33.27	0.0361	***
	425–474	–	–	–	

2.3.1 Discussion

Most treatment cell observations comprised a low number of tows (1–3), and as a consequence between-tow variation in mortality may not be well represented and the bootstrap analyses should be interpreted with caution. There was a strong inference that 12 mm PIP tags are unlikely to induce higher initial mortality in snapper than tags used in previous studies. There was some evidence that 23 mm PIP tag may induce higher rates of mortality in snapper than the 12 mm PIP tag and other tag types investigated.

3 PIP TAGGED SNAPPER THREE-MONTH HOLDING STUDY

3.1 Introduction

The medium-term effects of 12 and 23 mm PIP tags on snapper were assessed from tagged fish held in a sea-cage enclosure for 12 weeks. At the end of the holding period, histological, physiological, and heavy metal analyses were undertaken on tagged and control fish. The results of these analyses are presented in subsequent sections. A description of the holding study design is provided in this section.

3.2 Methods

Snapper used in the study were hatched and raised at Moana Pacific's snapper-rearing facility at Kawau Island in the Hauraki Gulf. The hatchery fish had been kept in sea-pens most of their lives and were about three years old when selected for tagging. Being voracious feeders, the snapper were easily caught by hook and line. All the experimental fish were captured in this way, and after tagging they were placed in a separate sea pen (8 x 8 x 5 m) for observation.

The experimental design consisted of two tagging treatments (12 mm and 23 mm PIP), each consisting of 100 fish each, plus a control group of 100 untagged fish. All fish were line-caught, then measured on a rubberised measuring board. Tags were injected into the fish's peritoneal cavity with an appropriate gauge hypodermic needle. The injection needles were reused, but disinfected between each use. The experimental fish were fed under the same regime with which they had been raised. The cage was inspected about every two days; dead fish were checked for tags and frozen for later study.

At the close of the experiment some of the surviving fish were removed from the sea-cage by baited hook capture, and were immediately killed by the iki-jime method. This was done so samples could be obtained before stress hormones had time to manifest in the blood. The net was removed from the water to recover the remaining fish. All recovered fish were visually assessed on the categorical scale outlined in Section 2.

3.3 Results

At the end of the three-month holding period there had been three confirmed mortalities (Table 5). The one fish unaccounted for may also have died; possibly the remains had been scavenged from the net. One of the confirmed dead fish was tagged with a 12 mm PIP. The remains of all three dead fish had been severely scavenged, that it was not possible to ascertain their condition at the time of death. None of the fish, recovered alive at the end of the holding period, had been assigned 'moderate' or 'severe' condition scores.

As there was a surplus of control fish at the end of the study, it is unlikely that any of the control fish died. The distribution of tag types across the dead fish is unknown. Assuming approximately equal mortality by tag type, the level of mortality induced by the tagging procedure was about 2%.

Table 5: Numbers of snapper released and recovered from the holding net by treatment class and recovery status.

Released	Total	Alive	Dead
23 mm PIP	100		
12 mm PIP	100		
Controls	100		
Total	300		
Recovered			
23 mm PIP	94	94	0
12 mm PIP	97	96	1
Controls	100	100	0
Unknown status – surplus controls	5	5	0
Unknown status – observed escaped (dropped)	1	1	0
Unknown status – unaccounted for	1	0.5	0.5
Unknown status – dead in net tag lost	2	0	2
Total	300	296.5	3.5

A subsample of 148 fish was line-captured from the net to provide tissue and blood samples for later analysis. The order in which fish were extracted from the net varied in relation to tag type (Figure 3).

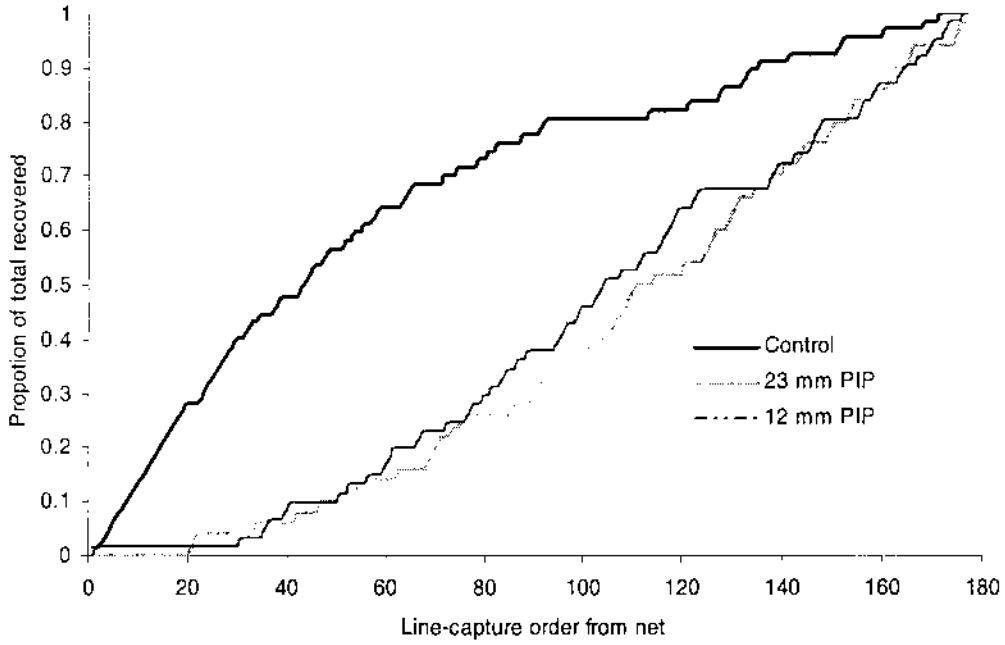


Figure 3: Snapper capture-order by treatment-type observed during collection of final sample.

3.3.1 Discussion

The reasons tags were missed upon recapture (there were five additional control fish) are unclear; only one failed (12 mm) tag was recovered. Possibly other tags had failed but inadequate scanning or scanner malfunction cannot be excluded as alternative explanations for missed tags.

This experiment was not specifically designed to investigate tag-failure; the tags used were early prototypes and their performance was not consistent. Results from specific tag-failure and performance analyses of tags made pursuant to the final manufacturing standards and specifications are presented in Sections 7 and 8.

The order in which fish were removed from the net during the second recovery varied between tag treatments. The observed treatment capture-order is consistent with a learning response, with the tagged fish being more 'cautious' of baited lines having being subjected to more 'invasive' handling during their initial capture. However, there may be other explanations. Whatever the reason, 'capture order' or 'recovery time' was not random across tag treatment classes (see Section 6).

4 HEAVY-METAL ACCUMULATION BY PIP TAGGED SNAPPER DURING THREE-MONTH HOLDING STUDY

4.1 Introduction and Methods

Excessive uptake of heavy metals by PIP-tagged snapper over the course of the three-month holding study would be evidence of tag-related chemical leaching. Heavy-metal assays of liver and body-wall tissue samples were analysed using General Linear Model (GLM) statistical procedures. The treatment classes investigated were '12 mm' and '23 mm' PIP tagged fish and 'untagged' controls.

Biomark Limited (confidential data) provided information on heavy-metal concentrations in the PIT electronics. Based on the Biomark data, heavy-metals of possible concern were: silver (Ag), aluminum (Al), copper (Cu), lead (Pb), tin (Sn), and zinc (Zn).

Flesh and liver samples from tagged and control fish (Table 6) were subjected to a hot concentrated nitric acid digestion (BDH Aristar grade reagents). Samples ranged between 0.7 and 6.7 g fresh weight, were digested to near dryness twice in 10 ml HNO_3, then taken up in 0.5 ml HNO_3 and made up to 10 ml total volume. Following centrifugation, trace metals in the digests were determined by inductively coupled plasma mass spectrometry (ICPMS) at R. J. Hill Laboratories Ltd in Hamilton, according to method APHA 3125B. Detection limits for the ICPMS analyses were 0.0001 g m^{-3} for Pb, 0.0005 g m^{-3} for Ag, Sn, and Cu, and 0.003 g m^{-3} for Al.

Table 6: Number of heavy-metal (Ag, Cu, Pb, Sn, Zn) assay samples by treatment class

	Flesh	Liver
Controls	5	5
23 mm PIP	5	5
12 mm PIP	8	6
Total	18	16

4.2 Results

The concentrations of heavy metals in the liver and flesh samples did not differ significantly between treatment classes (Table 7). It was concluded that there was no evidence of heavy-metal uptake in tagged fish over the three-month captivity period.

Table 7: Heavy metal assay mean concentrations and associated probabilities that concentrations differed between tagged and untagged controls.

	Flesh		Liver	
	(mean g m^{-3})	P< F	(mean g m^{-3})	P< F
Ag	0.0009	0.5639	0.0177	0.6923
Al	0.4587	0.1050	0.4426	0.8165
Cu	0.1428	0.1259	3.3325	0.9357
Pb	0.0495	0.8055	0.0562	0.1247
Sn	0.0174	0.8603	0.0077	0.7203
Zn	4.3398	0.1372	27.8354	0.9821

5 HISTOLOGICAL EVALUATION OF PIP-TAGGED SNAPPER FROM 3 MONTH HOLDING STUDY

5.1 Introduction and methods

Fish removed from the holding net by line-capture were evaluated and any gross lesions or ectoparasitic infections noted. The tissue response to the tag was then examined in a subsample of 20 fish from each tag treatment, and compared against the condition of comparable tissues taken from 10 control fish. For each tagged fish, three samples were taken, i.e., the peritoneal wall through which the PIT tag was inserted (tag insertion site), the tag lodgement site, and a sample of liver. These were

excised and fixed in 10% formalin buffered in filtered seawater. The tissues were processed for routine histology and stained with haematoxylin and eosin (H&E). The tissue responses in each of the samples were examined and the histopathological lesions observed were placed into 12 main categories (Appendix 2). Lesions classified as part of the normal wound repair response were given a lesion severity score of 0, unless there was evidence that they were unresolved or exaggerated, in which case they were scored as 2. Lesions, which were considered complicating factors during wound repair, were scored as 2, except when they were exaggerated or unresolved, when they were scored as 4. The presence of opportunistic bacteria, fungi, and myxozoa/protozoa were scored as 4, unless there was evidence of unresolved infection, when they were scored as 8 (Appendix 2). From these categories a cumulative tag acceptance score for tagged fish was devised (0–1, very healthy; 2–4, moderately healthy, 6–10, acceptable; 12 or more unacceptably compromised). The guidelines for acceptable lesions in tagged fish were based on the assumption that those control fish with myxozoan infections and existing normal inflammatory responses to these would be classed as moderately healthy (score 0–4). Any additional lesions above those observed in control fish would therefore be classed either as acceptable (score 6–10), or unacceptably compromised by the tagging procedure (12 or more).

5.2 Results

5.2.1 Grossly visible lesions

Very few fish had grossly visible lesions from tagging. Of the fish tagged with the 12 mm PIP tag, two (10%) exhibited minor melanisation at the tag entry site (Appendix 3), but in the remaining fish it was very difficult to ascertain the exact tag entry site because it had healed so well. Two fish (10%) tagged with the 23 mm PIP tag had either slight ulceration or bruising at the tag entry site (Appendix 4), but again it was difficult to ascertain the exact tag insertion site for the remainder of the fish tagged with this type of tag because the tag entry site had healed so well.

A large proportion of fish in both treatments and controls (55 %) of fish tagged with the 12 mm PIP tags, 75% of fish tagged with the 23 mm PIP tags and 90% of control fish, (Appendix 5) had numerous externally visible ulcerative, haemorrhagic lesions caused by infection with the ectoparasitic monogenean *Benedenia sekii* (see Figure 4 and Figure 5). In most of these fish between 1 and 37 *B. sekii* were evident on the external surfaces. Mean abundance of *B. sekii* on external surfaces ranged between 4.5 for fish tagged with 12 mm PIP tags, to 3.35 and 2.8 for fish tagged with 23 mm PIP tags and control fish, respectively (Appendix 3, Appendix 4, Appendix 5).

A green coloration of part of the liver was noted in two fish (one tagged with the 23 mm PIP tag, one control, Appendix 3 and Appendix 4). This was due to biliary stasis and is usually caused by bile duct blockage. In seacaged snapper this is usually due to infection of the bile ducts by plasmodia of a myxosporean parasite, *Myxidium sp.*, and was considered unrelated to the tagging procedure.

5.2.2 Tag lodgement locations

The 23 mm PIP tag was found encapsulated by adipose or connective tissue either low within the peritoneum near the vent (Figure 6), or adjacent to the gonad in all fish. The 12 mm PIP tags were also found encapsulated by adipose or connective tissue in these sites (but usually immediately adjacent to the gonad) in 75% of fish (Figure 7), but in the remaining fish the small tags were located either inside the gonad (3 fish), or in the muscle of the peritoneal wall (2 fish).

5.2.3 Pathological lesions

Two control fish were found to be infected by myxozoan parasites. One fish had large plasmodia in the bile ducts of the liver, which corresponded to the clearly evident green coloration of parts of its liver due to biliary stasis caused by bile duct blockage. The other control fish had myxozoan developmental stages in the epithelium of the rectum, which elicited a minor inflammatory response. There were no other notable lesions evident in control fish, all of which had normal epidermis, dermis, hypodermis, muscle and adipose tissue (Figure 8 and Figure 9).

Fish tagged with both the 12 and 23 mm PIP tags generally showed normal wound repair responses at the tag entry and tag lodgement sites. Normal wound repair was associated with minor epithelial hyperplasia and epithelialisation over the original wound site and tag sinus, resolved inflammation (mainly macrophages, eosinophillic granular cells and lymphocytes which often showed tracking through the dermis, hypodermis and muscle) (Figure 10), and fibrosis of the tissues damaged by the tagging needle. Degeneration of muscle fibres (myodegeneration) was often evident in and surrounding the tag sinus (Figure 11). At the tag lodgement, site evidence of focal, chronic inflammation and fibrotic encapsulation of the tag by adipose and connective tissues was noted (Figure 12). The most common complicating lesion recorded at the tag lodgement site was extensive inflammation, and inflammatory cell tracking, which were considered to be exaggerated normal responses (Figure 13). Haemorrhage and necrosis of the tissues adjacent to the tag and tag sinus were rare.

The most common abnormal complication observed in fish tagged with both tag types was granuloma formation which was usually associated with either fungal or occasionally bacterial infection. Granulomas were recorded in four fish (20%) tagged with the 12 mm tag. In these fish granulomas in the dermis and muscle at the tag entry site (two fish) (Figure 14) were associated with the presence of bacteria, while granulomas in adipose tissue at the tag lodgement site (two fish) (Figure 13) were associated with both bacteria and fungi. Granulomas evident at the tag entry site in 25% of fish tagged with the 23 mm tag were mostly associated with the presence of fungi in the dermis, hypodermis (Figure 15), and muscle. The prevalence of granulomas in adipose and connective tissue at the lodgement site of 23 mm tags was 35%. Granuloma at the tag lodgement site were associated with the presence of fungi in one fish, but in the other fish tagged with the 23 mm tag the cause of the granulomas was unclear.

Lesions in the liver of all fish were restricted to those associated with the Myxosporean *Myxidium sp.*, which was recorded in 20% of both control fish and fish tagged with the 12 mm tags, and 10% of fish tagged with the 23 mm tag.

When the overall lesion scores were compared between fish with small and large tags, mean lesion scores were 3.7 and 3.5 (moderately healthy) for small and large tags, respectively (Appendix 3 and Appendix 4). The mean lesion score for control fish was 0.8 (very healthy; Appendix 4). Only one fish gained an unacceptable (over 12) total lesion score. That fish was double tagged with large tags and obtained a total score of 16 consisting of a score of 12 at the tag lodgement site due to the presence of granulomas, bacteria, and fungi and an extensive inflammatory response, plus a score of 4 for the liver due to the presence of Myxidium sp. in the bile ducts.

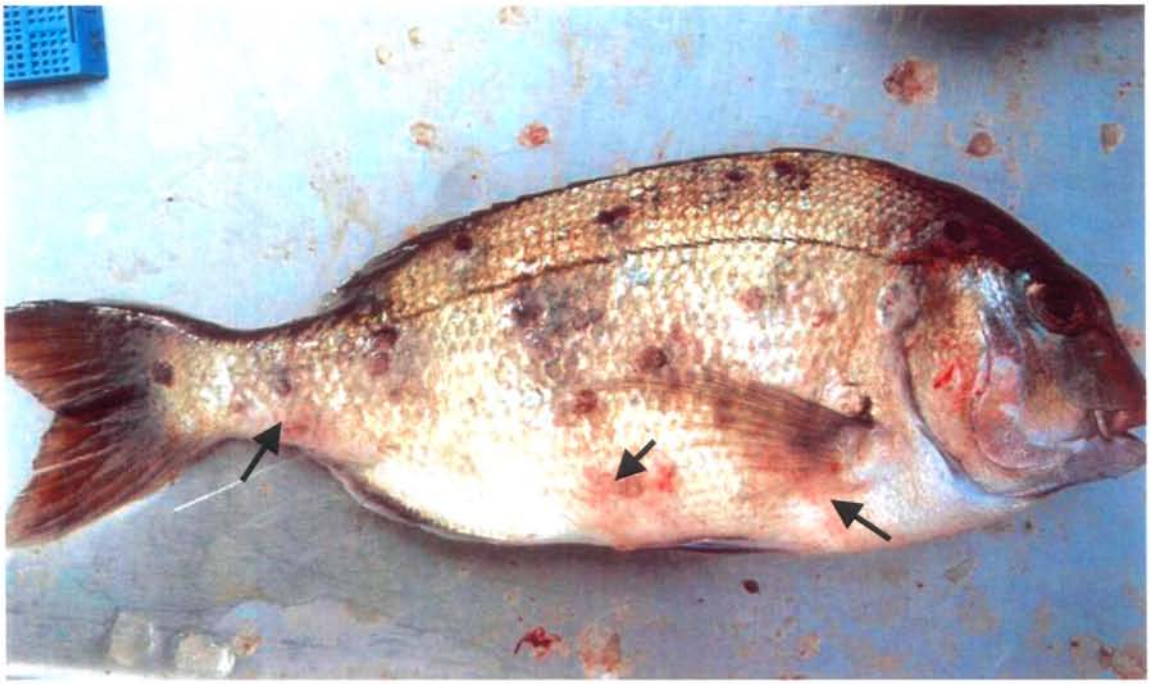


Figure 4: **Tagged snapper infected with numerous monogeneans (*Benedenia sekii*), which appear as dark coloured round dots on the flanks. Note also the haemorrhagic ulcerative lesions in areas where the parasites were, indicated by arrows.**

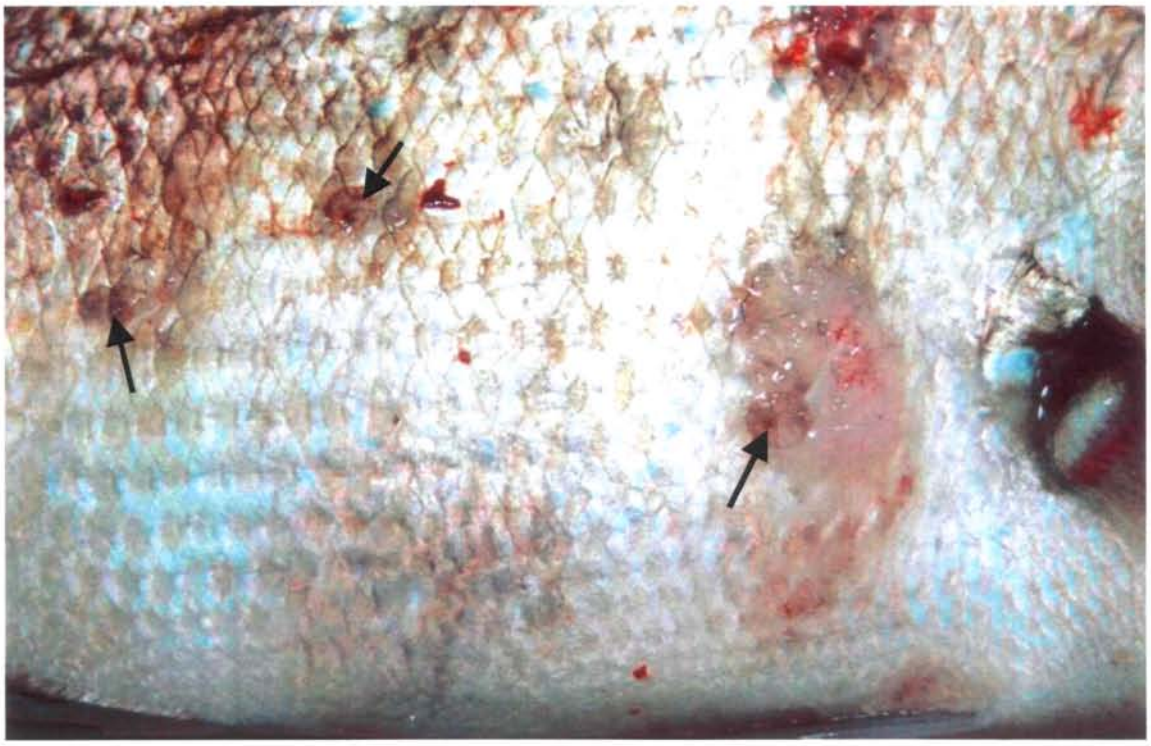


Figure 5: **Closer view of lesions on the flanks of caged snapper associated with presence of the monogenean *Benedenia sekii* (arrows).**

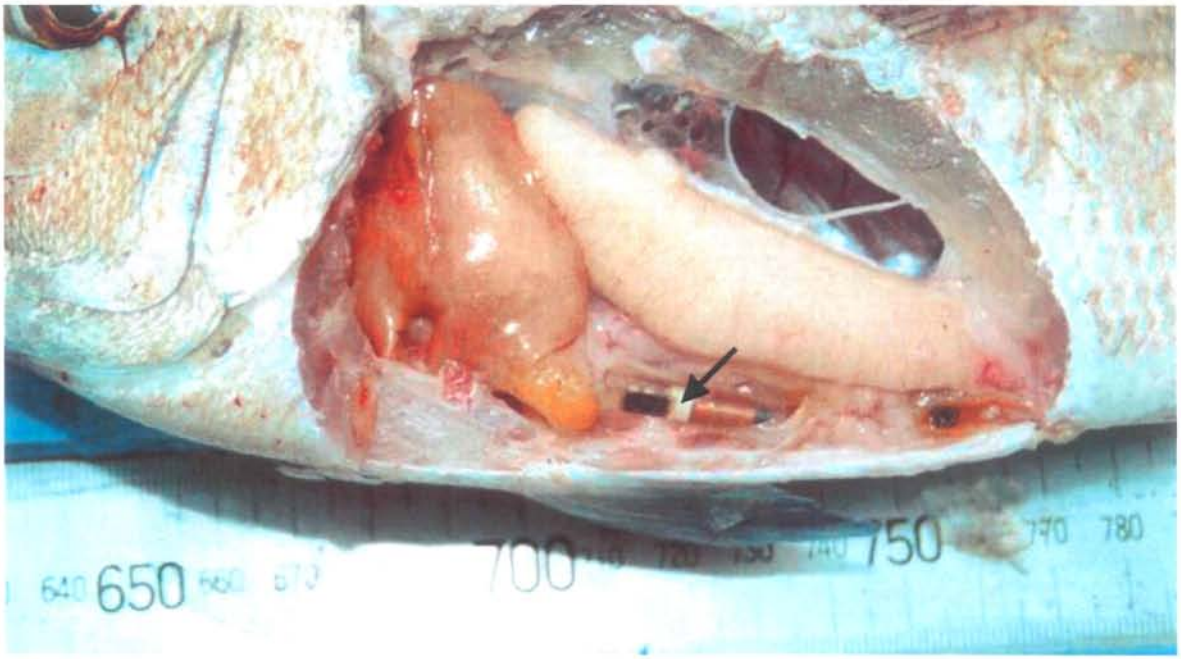


Figure 6: **Gross appearance and typical position of the tag lodgement site for a 23 mm PIP tag (arrow), namely low in the peritoneum, ventral to the gonad, and entrapped by the adipose tissue in this female snapper.**

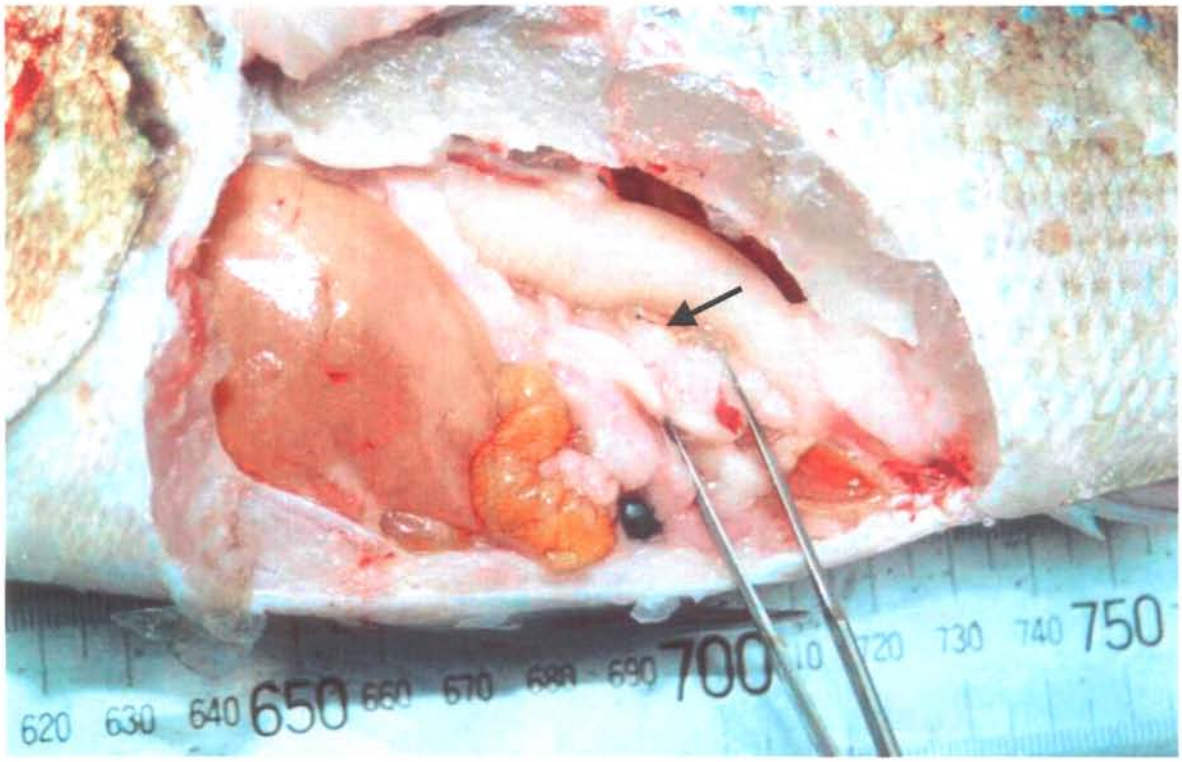
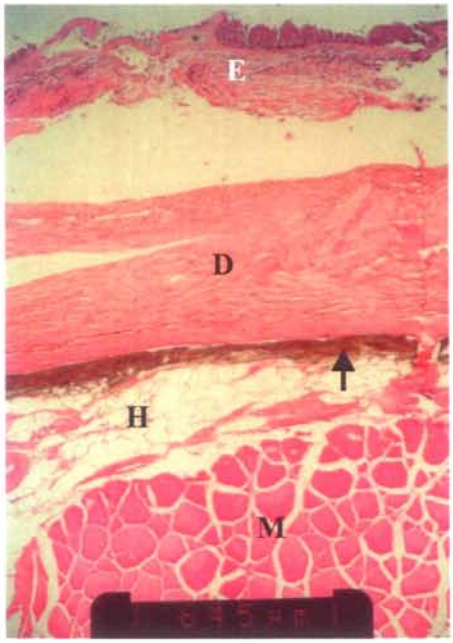


Figure 7: **Gross appearance and typical position of the tag lodgement site for a 12 mm PIP tag (arrow), namely embedded in the adipose tissue immediately adjacent to the gonad in this female snapper.**



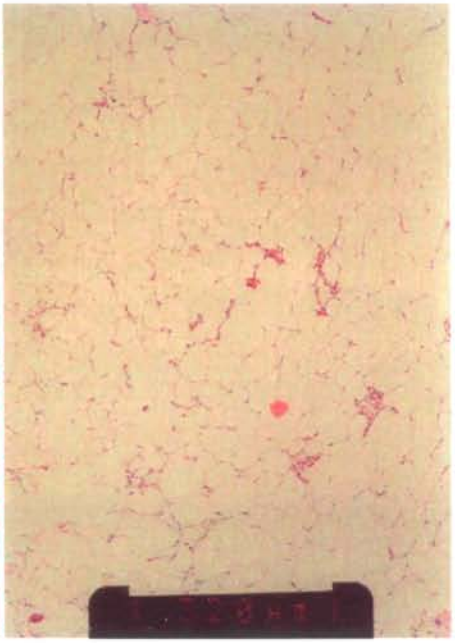
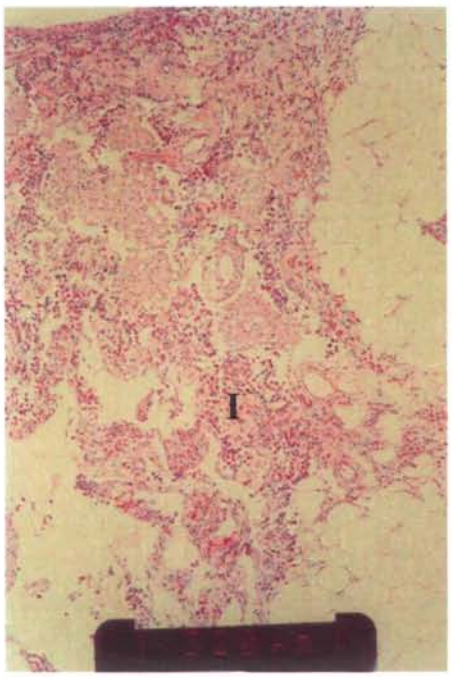


Figure 8 (above, left): **Histological section through the skin of a control fish showing normal epidermis (E), dermis (D), hypodermis (H) containing a layer of pigment (arrow) and underlying muscle (M).**

Figure 9 (above, right): **Histology of normal adipose tissue.**



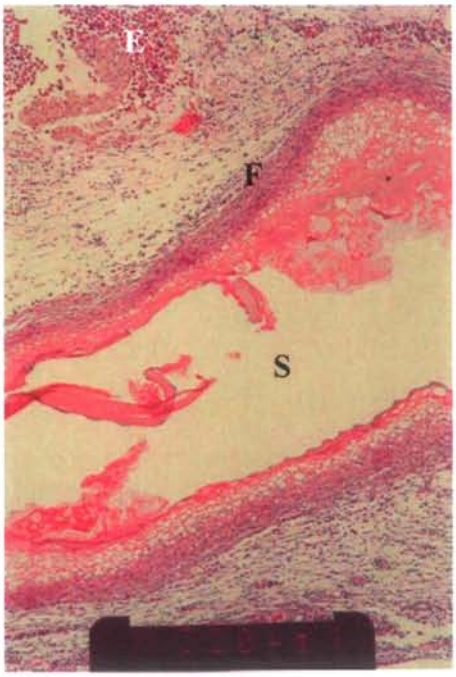


Figure 10 (above left): **Histology of normal inflammation at a tag lodgement site in adipose tissue adjacent to a small tag. Note infiltration of many inflammatory cells (I), mostly eosinophillic granular cells and macrophages, and fibrosis.**

Figure 11 (above right): **A longitudinal section through the tag lodgement site for a large tag, showing an eosinophillic layer immediately adjacent to the tag sinus (S), which is surrounded by a capsule of fibrous tissue (F) laid down by inflammatory cells, including numerous eosinophillic granular cells (E).**

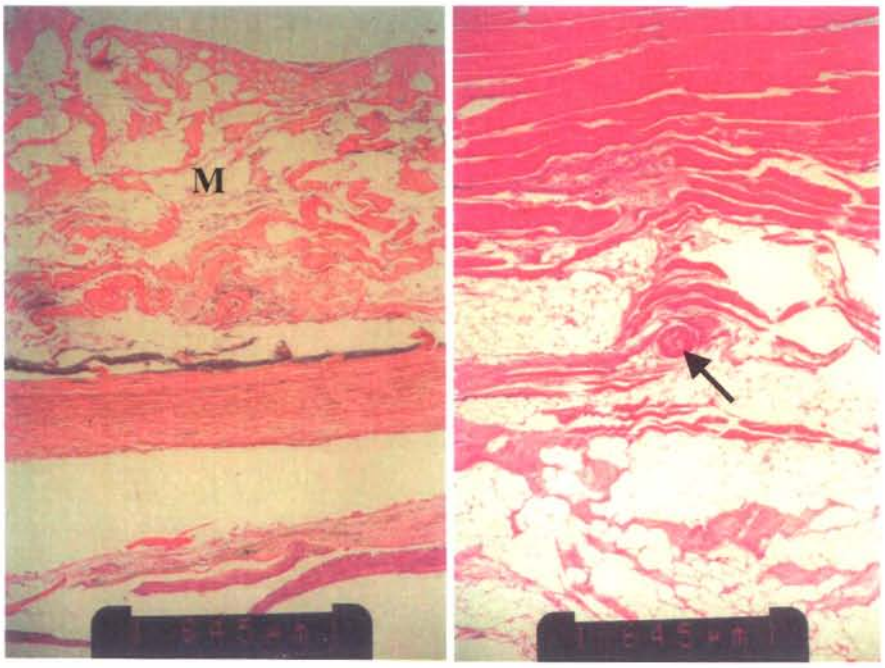


Figure 12 (above left): An area of myodegeneration (M), inflammation and fibrosis of muscle adjacent to a tag sinus.

Figure 13 (above right): A granuloma (arrow) between the hypodermis and muscle near a tag entry sinus.

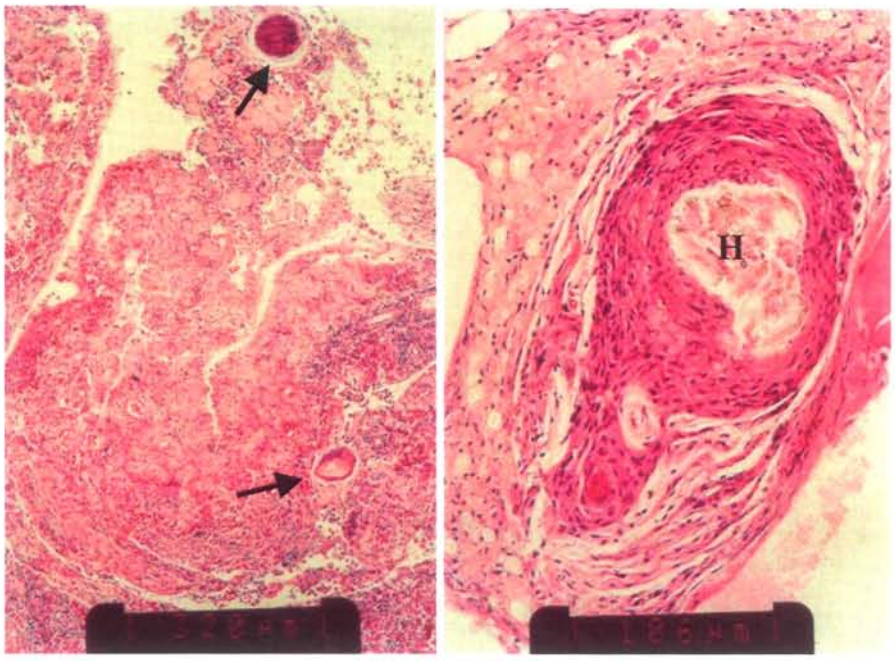


Figure 14 (above left): Extensive inflammation and fibrosis, including 2 granulomas (arrows) at the tag lodgement site of a large tag.

Figure 15 (above right): Higher magnification of a granuloma in the hypodermis of a fish tagged with a large tag. Note necrotic fungal hyphae (H) in the centre of the granuloma.

5.3 Discussion

The tagging procedure for both tags resulted in externally visible lesions in 10% of fish. The most obvious of these were not associated with the tagging procedure, but were due to infection with the monogenean ectoparasite *Benedenia sekii*. This parasite occurs naturally on snapper in New Zealand (Roubal et al. 1983, Sharples & Evans 1995), but tends to build up on fish confined in seacages (Roubal et al. 1996, Diggles et al. 2001). The normal presence of similar parasite-associated lesions on wild fish would appear to make it difficult, if not impossible, for commercial and recreational fishers to detect tagged fish from simple external examination, at least after 12 weeks post-tagging.

The vast majority of fish examined histologically showed myodegeneration, epithelialisation, inflammation, and fibrosis of the tag entry site. These are all normal wound repair responses to tag entry wounds (Mawdesley-Thomas & Buke 1973, Roberts et al. 1973b). Recovering of the tag sinus by epithelium occurs quite quickly in fish, a process that minimises the chances of secondary infection (Roberts 1989). Once the epithelial migration over the wound restores the osmotic and infection barrier, regeneration of the dermis and underlying muscle takes longer and is slowed by low temperatures (Roberts 1989). This might suggest that tagging should be undertaken in summer whenever possible as higher water temperatures are likely to promote faster wound healing, though ideally the effect of temperature on wound repair in snapper should be studied to confirm this. In the absence of such information, a logical decision would be to use 12 mm tags with their smaller tagging needles wherever possible to reduce the size of the tag entry site.

The tags did not appear to cause undue irritation to internal organs. At the tag lodgement site, most tags were encapsulated by adipose or connective tissue with little or no host inflammation evident to the naked eye. Even with histology, few notable complicating lesions were evident in fish. Only one fish accumulated an unacceptably high total lesion score. The greater than normal inflammatory response at the tag lodgement site in this fish may have been due to the double tagging procedure which it endured, which probably doubled its chances of secondary bacterial and fungal infection[1]. However, even for the double-tagged fish, the presence of either type of tag caused no significant changes to the normal appearance of the internal organs upon gross examination. This suggests that both sizes of PIP tags are well tolerated by snapper and have little influence on the appearance or function of their internal organs.

The most common complicating lesion observed microscopically was granuloma associated with secondary fungal infection of the tag entry and lodgement sites. Granuloma formation is a common complication of the tagging process in a variety of fishes (Roberts et al. 1973c, Vogelbein & Overstreet 1987), and is usually associated with chronic infection of the wound by opportunistic bacteria and/or fungi. Soaking the tags or swabbing the tag entry site with an antiseptic solution such as iodine, and/or more regular sterilisation of tagging equipment, might have reduced the low prevalence of fungal infections observed in the present study even further.

From a pathological point of view, both 23 and 23 mm PIP tags were equally well accepted by the fish, hence the decision as to the most appropriate tag type can be based on logistical criteria other than the type of response of the fish to the tags. If other criteria are inconclusive, use of the 12 mm tags is preferred due to reduced physical damage to the peritoneum (due to the smaller tag sinus), which may reduce the chances of secondary infection. However, this must be balanced against the fact that the 12 mm tags were more likely to migrate from the tagging site and lodge in the gonad, or embed in the wall of the peritoneum, than were the large tags. Conversely, the large tags would be easier for commercial and recreational fishers to detect, but only once fish were opened for gutting.

[1] The fish received a second tag because the first had failed to operate. None of the other experimental animals were double-tagged.

6 PHYSIOLOGICAL RESPONSES TO CHRONIC STRESS IN PIP TAGGED SNAPPER

6.1 Introduction

It is inevitable that fish will exhibit some degree of stress response to capture and handling. The adaptive nature of this response is to mobilise energy substrates and increase cardiovascular and oxygen transport capacity, enhancing the scope for physical activity under threat. However, the disturbance of the hydromineral balance and accumulation of exercise by-products under extreme, acute stress can become maladaptive and result in mortality when coping mechanisms are forced beyond tolerable physiological limits. Persistent long-term, or chronic, stress may not necessarily cause mortality, but can inhibit growth, reproductive ability and immunocompetence, and reduce the capacity to tolerate additional stressors. These effects of chronic stress can have detrimental consequences at the population level.

Stress responses manifest as a variety of integrated biochemical events that may appear within seconds and can persist for hours or even days after exposure to a stressor. In teleosts the primary response following endocrine stimulation involves the release of catecholamines (adrenalin and noradrenalin) and corticosteroids, mainly cortisol, from the chromaffin and interrenal cells of the head kidneys respectively (Wendelaar Bonga 1997). While catecholamines play an integral part in the physiological stress response by rapidly increasing ventilation rate, oxygen transport capacity, and stimulating hepatic glycogen metabolism, they also fluctuate very quickly, which makes them difficult to monitor. Cortisol, which regulates hydromineral homeostasis and affects lipid, protein, and carbohydrate metabolism is generally considered a reliable index of the physiological response to stress in fish because resting levels are typically low (Pankhurst & Sharples 1992, Wendelaar Bonga 1997). The subsequent secondary responses that follow this hormonal cascade include perturbations in blood glucose concentration, plasma protein, and haematological parameters. The magnitude and extent to which these physiological parameters alter are determined by the duration and severity of exposure to a particular stressor.

6.2 Methods

To assess the occurrence of chronic stress in PIP-tagged snapper, a range of biochemical stress response parameters was measured in tagged and untagged fish at the end of the 12-week holding period (Table 8). For each parameter measured baseline measurements were obtained from a separate group of 100 snapper before the holding study began. After providing baseline data, these fish took no further part in the experiment.

At the end of the 12-week holding study snapper were removed from the net using baited-lines and about 1 ml of blood was immediately collected using a heparinised 25 g needle inserted in the caudal vein. Blood glucose was measured by placing a drop of blood on to the test strip of a portable glucose meter (Advantage; Roche Diagnostics, Switzerland). To measure haematocrit (Hct) and leucocrit (Lct), blood was drawn into a standard microhaematocrit capillary tube, plugged with modelling clay, and centrifuged for 5 minutes at 3000 rpm. Hct and Lct were then measured using an ocular micrometer on a dissecting microscope. Haemoglobin (Hb) concentration was assayed by reaction of 10µl of blood with Drabkin's reagent; absorbance was then measured in a spectrophotometer at 540 nm. Mean cell haemoglobin concentration (MCHC) was derived from [Hb]/(Hct/100). Plasma was then separated from the remainder of the blood by centrifuge (as above) in 1.5 ml eppendorf tubes. Protein was assayed by placing 20 µl of plasma on a hand-held protein refractometer that had been zeroed using distilled water. The remainder of the plasma was then stored at –80 °C for later cortisol analysis. Plasma cortisol concentrations were determined using the radio-immunoassay (RIA) protocol given by Pankhurst & Conroy (1987).

Table 8: Description of biochemical parameters measured during this study, and criteria for evaluating the severity of the stress response, criteria developed from Pankhurst & Sharples (1992), Lowe & Wells (1996), and Wells & Pankhurst (1999).

Stress parameters	Stress indicator	Physiological consequence of altered stress parameter	Criteria used to evaluate the stress response
Cortisol	Acute and chronic	Corticosteroids regulate energy metabolism & hydromineral balance. Chronic cortisol elevation depresses immune function and reproductive capacity.	Acute levels > 70 ng/ml. Chronic levels significantly higher than baseline levels.
Glucose	Acute	Glucose is a primary metabolic energy substrate. Prolonged hyperglycaemia depletes stored metabolic energy, making it unavailable for growth and reproduction.	Acute levels >12 mmol/l
Hematology	Acute and chronic	Increased mean cell haemoglobin concentration (MCHC) indicates haemodilution which reduces oxygen transport capacity. Decreased haemoglobin concentration (Hb) and haematocrit (Hct) indicates potential anaemia.	Acute: MCHC significantly higher than baseline levels. Chronic: Hb and Hct significantly lower than baseline levels.
Leucocrit	Chronic	A reduction in circulating leucocytes leads to reduced immunocompetence.	Chronic levels significantly lower than baseline levels.
Plasma protein	Chronic	Under extreme physical or nutritional stress, plasma protein is markedly increased, which can impair growth rates, osmoregulation and the immune response.	Chronic levels significantly higher than baseline levels.

A General Linear Model (GLM) was used to assess variability in the biochemistry between the two tag-size groups and the control fish (= 0.05). The effects of order of removal from the net (ORDER) and treatment (TREATMENT) were investigated in the GLM. The values for each biochemical parameter were logged before fitting the GLMs.

A GLM was also used to assess variability in the biochemistry between the experimental groups and the baseline group (= 0.05). The initial untagged readings were sampled from the net over a 2.5 hour period, the time of removal being known for each fish. Time/order of removal from the net was found to influence many of the stress parameters measured. Consequently, a subset of the experimental fish was obtained using only fish removed during the first 2.5 hours of sampling. Values for the initial group were combined with the experimental groups to draw comparisons between the baseline level of stress parameters and fish involved in the tagging experiment, with TIME being elapsed time from the start of removals each day. The resulting data set although highly unbalanced in favour of the baseline levels provides some interesting comparisons nevertheless (Table 9). The effects investigated in the GLM were TREATMENT and TIME since removal began. The values for each biochemical parameter were logged before fitting the GLMs.

Table 9: Sample sizes of experimental subsets used for comparison of treatment stress parameters with initial/baseline levels.

Treatment	n
INITIAL	100
CONTROL	49
LARGE (23 mm)	13
SMALL12 (12 mm)	17
Total	179

6.3 Results

6.3.1 Comparisons between tagged and untagged fish

No significant differences were found between the means of any of the stress parameters from the tagged or untagged fish, although capture order had a significant effect on glucose, haemoglobin, and leucocrit concentrations (Table 10). These parameters were typically elevated in fish removed from the net early on the day of capture (Figure 16 and Figure 17). For cortisol, the 'treatment/order' interaction was significant (Table 10), meaning the influence of tag treatment varied in relation to the order fish were removed from the net. Cortisol concentrations were generally higher in the untagged group, although these elevated responses were observed earlier in the day, when most of the untagged fish were caught (Figure 16).

Table 10: Comparisons of biochemical stress parameters between PIP-tagged fish and control fish. Treatment contained three groups of fish; small12 (12 mm PIP), large (23 mm PIP) and control (no tag). Order represented the capture order from the net. Comparisons significant at the 5% level are represented by '*'; NS, not significant; NA, not applicable due to significant interaction effect.**

Parameter	Treatment	Order	Interaction effect	Treatment mean	Treatment S.E.
Cortisol ($ng.ml^{-1}$)	NA	NA	***	NA	NA
Glucose ($mmol.l^{-1}$)	NS	***	NS	2.15	0.04
Haematocrit (%)	NS	NS	NS	23.88	0.52
Haemoglobin ($g.l^{-1}$)	NS	***	NS	44.81	1.66
MCHC ($g.l^{-1}$)	NS	NS	NS	311.11	6.81
Leucocrit (%)	NS	***	NS	1.29	0.04
Protein ($g.dl^{-1}$)	NS	NS	NS	5.34	0.05

6.3.2 Comparisons between experimental snapper and baseline levels

Each of the three groups of snapper from the tagging experiment demonstrated some degree of elevated stress response compared to baseline levels (Table 11). An acute stress response was evident in the significantly elevated Mean Cell Haemoglobin Concentration values in all three groups, which reflects the magnitude of changes in haemoglobin concentration relative to packed erythrocyte volume (haematocrit). Some degree of chronic stress was detected in all three groups as evidenced by a significant reduction in haematocrit, while haemoglobin concentration remained unchanged in all but the large PIP tag group, where it was significantly lower than baseline.

Significant differences in the other indicators of chronic stress (plasma protein, cortisol, and leucocrit) were also observed between some of the experimental fish and baseline levels (Table 11). Elevated plasma protein and cortisol concentrations were observed in the control and small tag groups, and control and large tag groups, respectively. An increase in leucocrit occurred in the control group, yet no difference was found in this parameter between both of the tagged groups and the baseline.

The 'time/treament' interaction term was again significant in the cortisol GLM, meaning the relative differences in the treatment cortisol readings varied depending upon the time of removal from the net. For this reason the mean cortisol values given in Table 11are not strictly comparable statistically.

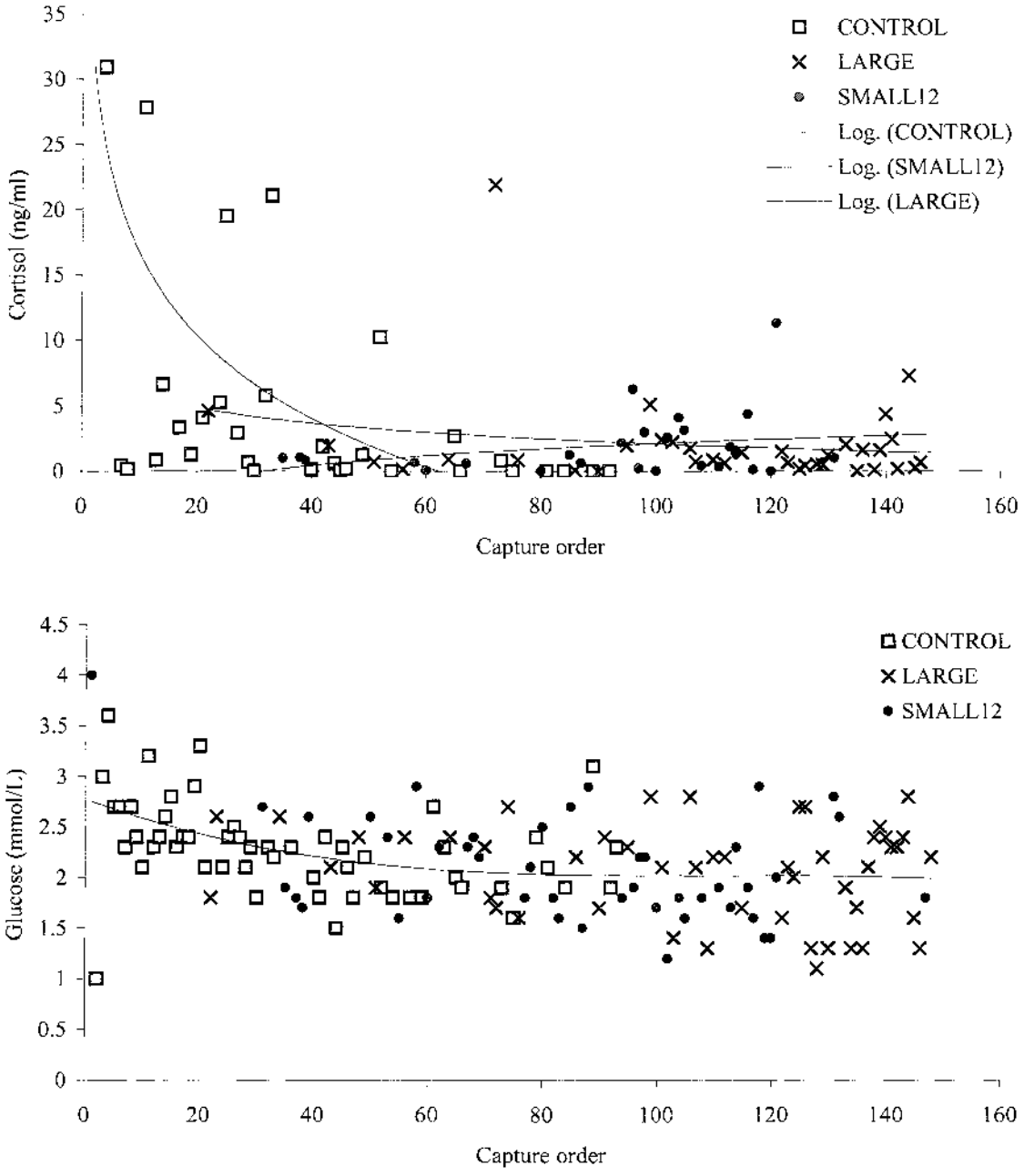


Figure 16: Relationship between plasma cortisol, blood glucose concentrations and capture order in snapper used to evaluate PIP tags with log-linear least-squares regression lines. Control = no tag; small = 12 mm PIP tag; large = 23 mm PIP tag.

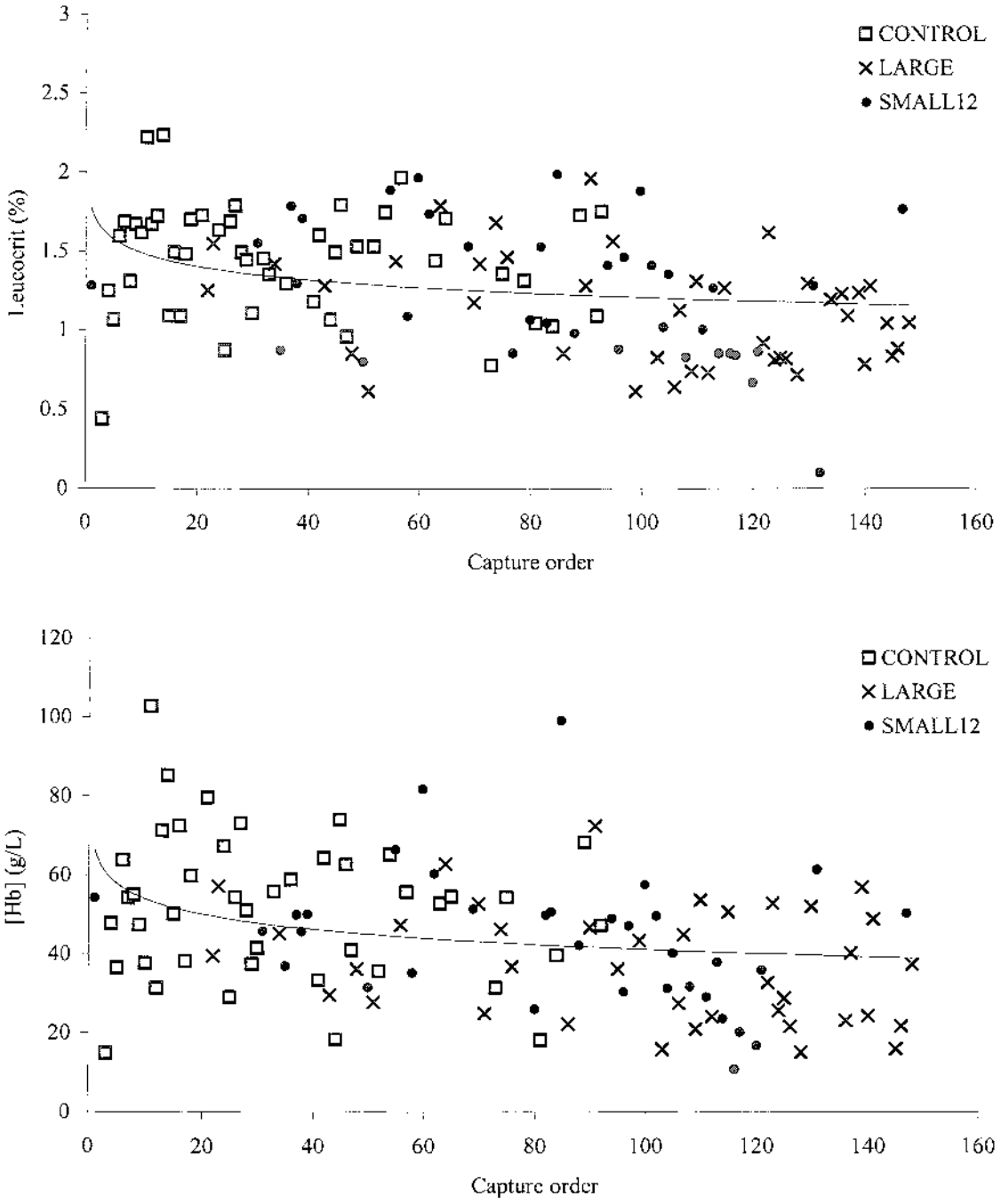


Figure 17: Relationship between leucocrit, haemoglobin concentration and capture order in snapper used to evaluate PIP tags with log-linear least-squares regression line. Control = no tag; small = 12 mm PIP tag; large = 23 mm PIP tag

Table 11: Biochemical stress parameters (mean ± S. E.) from "baseline" snapper and snapper used to evaluate PIP tags (small = 12 mm PIP tag; large = 23 mm PIP tag; control = no tag). '*' indicates values that are significantly different from baseline at the 5% level.**

Parameter	Baseline	Control	Small	Large
†Cortisol ($ng.ml^{-1}$)	0.6 ± 0.2	7.5 ± 2.7 ***	0.7 ± 0.2	4.4 ± 3.0 ***
Glucose ($mmol.l^{-1}$)	2.1 ± 0.1	2.3 ± 0.1 ***	2.3 ± 0.1	2.2 ± 0.1
Hematocrit (%)	32.7 ± 1.4	25.2 ± 0.8 ***	24.3 ± 1.0 ***	22.4 ± 1.7 ***
Haemoglobin ($g.l^{-1}$)	53.8 ± 1.4	52.8 ± 2.9	50.8 ± 4.1	42.2 ± 3.4 ***
MCHC ($g.l^{-1}$)	176.6 ± 8.5	307.7 ± 11.5 ***	284.9 ± 13.8 ***	335.1 ± 17.8 ***
Leucocrit (%)	1.3 ± 0.1	1.5 ± 0.1 ***	1.4 ± 0.1	1.3 ± 0.1
Protein ($g.dl^{-1}$)	4.9 ± 0.1	5.3 ± 0.1 ***	5.5 ± 0.1 ***	5.4 ± 0.2

† Significant 'Time-Treatment' interaction in GLM; treatment means are not strictly statistically comparable

6.4 Discussion

Two main outcomes were derived from the observation of stress biochemistry in snapper over the 12-week holding study. Firstly, snapper appeared indifferent to tag placement or the type of tag used, as evidenced by the lack of negative differences between the physiological profiles of untagged snapper and those with 12 or 23 mm PIP tags. Secondly, elements of the experimental procedure, most likely line capture and handling, caused some degree of chronic stress response (according to the criteria listed in Table 10) in both tagged and untagged fish. The most consistent biochemical marker that reflected this response across all three groups was a general reduction in haematocrit, although elevated plasma protein also indicated chronic stress exposure in most groups; the failure to detect a difference in plasma protein between the baseline and large PIP tagged group was most likely due to the unbalanced sample sizes. This sample size artefact and the influence of capture time/order made it difficult to clearly interpret changes in the other chronic stress parameters. Of the detectable changes, the most notable were the slight increases in cortisol concentration in the untagged and large PIP tagged fish, and the decreased haemoglobin concentration that was observed in the large PIP tag group.

Although cortisol concentrations were significantly elevated in two groups of snapper compared to baseline levels in the holding study, these values were similar to resting levels for Hauraki Gulf snapper published elsewhere. Pankhurst & Sharples (1992) established that resting cortisol concentrations in snapper ranged between 1.7 $ng.ml^{-1}$ in summer and 8.0 $ng.ml^{-1}$ in winter, which then increased to between 42 and 70 $ng.ml^{-1}$, 1 h and 3 h after exposure to a variety of fishing stressors respectively. A similar study recorded a mean cortisol concentration of 3.8 $ng.ml^{-1}$ from 10 snapper sampled underwater (Bollard et al. 1993). Unfortunately no data were available for snapper during the hours following tag implantation in the present study. Published data suggest that cortisol concentrations do not become significantly elevated in this species until about 1 h after exposure to a stressor. In this case, the cortisol data obtained from the holding study would be expected to represent resting levels and not the peak concentrations caused by acute stressors, i.e., capture, handling, and tag implantation. These data do indicate that the fish were able to attain a reasonable state of recovery by the end of the holding period, however. It is important to realise the implications this under-representation of cortisol elevation may have, as even short-term elevations of corticosteroids are believed to be responsible for depression of reproductive ability (Carragher & Pankhurst 1991). Extensive gonadal atresia has been observed in snapper following capture stress, which led some to believe that reproductive activity was likely to cease for the remainder of the spawning season and unlikely to resume until the following season (Pankhurst & Sharples 1992). A similar suppressive effect of corticosteroids on immunocompetence has also been recognised (Pankhurst & Sharples 1992).

The action of stress on suppressing the numbers and function of circulating lymphocytes (white blood cells involved in cell mediated and humoral immunity) is a major factor influencing disease resistance in fish (Barton & Iwama 1991). Experience in salmonid aquaculture has indicated that a single, 2 minute handling stressor was able to cause significant lymphocytopenia in brown trout that took up to 72 h to recover (Pickering et al 1982). No reduction in snapper leucocrit occurred during the holding study, although an increase was observed in the untagged fish. The cause of this increase is unknown, although it may reflect a minor relative improvement in immunocompetence that had not occurred in the tagged fish. Further trials involving pathogen challenges would be necessary to establish whether any real difference exists between the disease resistance profiles of tagged and untagged fish; the current data indicate that any such effect would be minimal.

Red cell haematology has found to be a useful stress indicator, but is often difficult to interpret because fish do not defend a constant haematocrit (Wells & Pankhurst 1999). Under stress, fish haematocrit increases as erythrocytes are released from the spleen and cell swelling occurs, which increases the animals' oxygen carrying capacity. In light of this response, both haemoglobin concentration and haematocrit values need to be considered together to evaluate the occurrence of potential anaemia. Although a marked reduction in haematocrit occurred in both tagged and untagged fish in the present study, only fish tagged with the large PIP tag showed a significant reduction in haemoglobin concentration. Together, these results indicate the presence of mild anaemia in the fish tagged with the larger tag, a response not observed in the other groups. This mild anaemia could result in a reduced oxygen transport capacity and potentially reduce the ability of these fish to sustain aerobic activity under additional stress or threat.

Plasma protein measurements provide a useful indicator of extreme physical stress, and increase where internal tissue damage or nutritional stress has occurred (Wells & Pankhurst 1999). A variety of stress proteins are produced following exposure to a stressor, and these have been implicated in cellular restoration, cellular protection, and increased stress tolerance. The fact that plasma protein was elevated in the untagged fish in the present study indicates that this response was once again due to the experimental procedure as a whole rather than tag implantation or the type of tag used.

Further effects of the stress caused by capture, handling, and tagging, such as inhibitory effects on reproductive hormone levels, gamete development and quality, and subsequent egg and larval survival should be investigated further. In light of evidence obtained during the holding study, it is believed that PIP tag implantation itself has little or no significant overall effect on fish health. It is recommended that future studies be conducted on fish with developing or ripe gonads to fully establish tagging effects on reproductive health.

7 EXTREME PRESSURE AND CHEMICAL EXPOSURE TRIALS

7.1 Introduction

The process leading up to the final PIP design involved extensive 'torture' testing of prototype designs. In this section we present results from the concluding series of tests intended to test the robustness of the final coated design. Tests were carried out only on the 12 mm PIP as it had been decided to use this tag for the 2002 SNA 8 tagging programme.

The two main stresses on a PIP embedded in a fish are pressure and chemical damage from body fluids. Tests were devised to investigate tag performance at the more extreme bounds of these two influences.

Although snapper are found off the west coast of the North Island out to depths of 200 m (21 atmospheres) most of the SNA 8 population is distributed across depths shallower than 75 m (8.5 atmospheres) (Drury 1993, Langley 1995, Morrison 1998, Morrison & Parkinson 2001). It was reasoned that if the PIP tags could withstand variable pressures up to 20 and 40 atmospheres for 3

weeks, the tags should be capable of surviving much longer under the lower pressures experienced by most of the SNA 8 stock.

7.2 Methods

The basic experimental design consisted of subjecting the 12 mm PIP tag and the 12 mm glass coated Destron TX 1400L tag to 20 and 40 atmosphere pressure regimes with controls. Tags were subjected to the pressure treatments for 3 weeks. The tag chambers were fully depressurised every 2 days at a fixed rate (10 atmospheres per minute) and readings were obtained for all tags every 7 days giving a total of four reading events. The experimental design is given in Table 12.

Table 12: Pressure trial experiment design showing treatment classes, level of replication, and frequency of testing.

Treatment	Tag-type	Exposure (days)			
		0	7	14	21
20 atm	GLASS	74	74	74	74
40 atm	GLASS	75	75	75	75
Control	GLASS	45	45	45	45
20 atm	PLASTIC	100	100	100	100
40 atm	PLASTIC	100	100	100	100
Control	PLASTIC	29	29	29	29

Tags were sealed in plastic sachets with a cocktail of isotonic fluids and fish oils designed to replicate the chemical effects of body fluids.

The maximum detection distance of each tag orientated vertically under an AllFlex™ 6 volt Stick Reader, termed the Maximum Vertical Distance Score (MVDS), was recorded (Figure 18). General Linear Model (GLM) procedures were used to analyse the MVDSs relative to the categorical variables 'Tag-type', 'Treatment', and 'Reading-event' (Table 12). The GLMs was used to test crossed effects (interactions) as well as main effects.

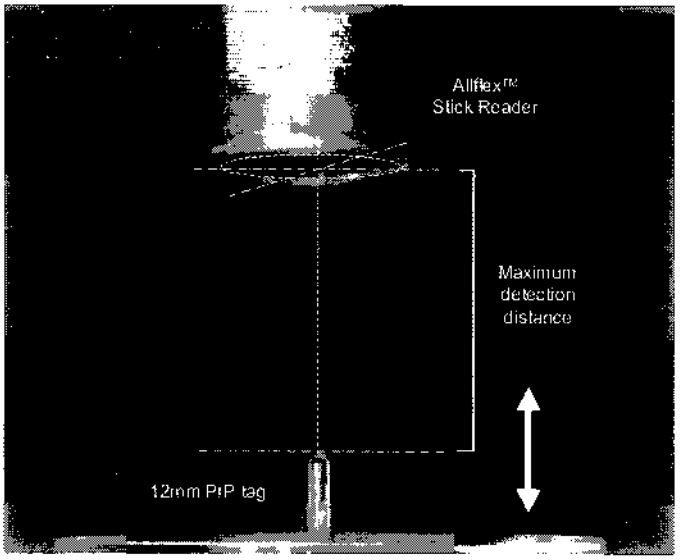


Figure 18: 12 mm PIP vertically aligned under AllflexTM Stick Reader for determining the maximum detection distance.

7.3 Results

7.3.1 Glass PIT GLMs

None of the glass experimental tags failed during the course of the experiment.

The interaction between 'Reading-event' and 'Treatment' was not significant in the mixed model GLM ($P < F = 0.1970$). In the main-effects GLM, 'Reading-event' was explanatory ($P < F = 0.0001$) but 'Treatment' was not ($P < F = 0.9436$). The Tukey test for differences in 'Reading-event' MVDS means revealed that the initial 'Reading event' mean was significantly higher than subsequent 'Reading event' means (Appendix 6; Figure 19).

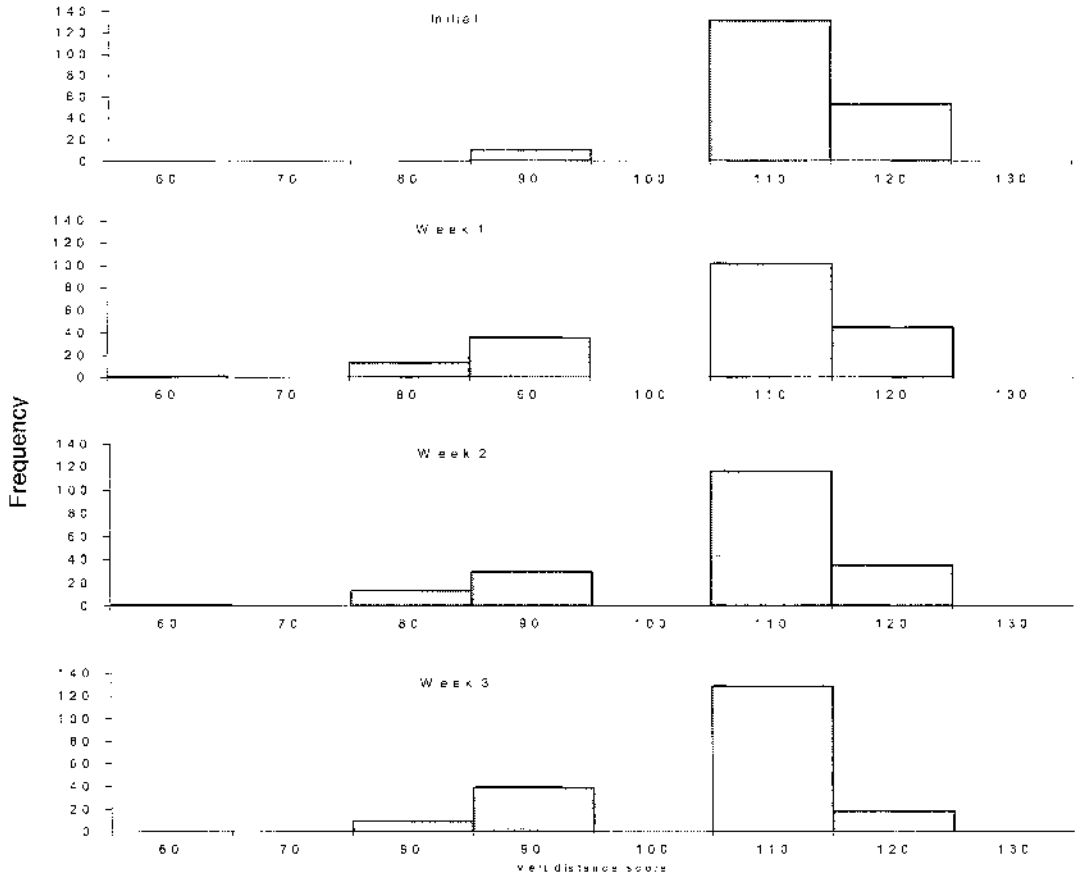


Figure 19: Weekly frequency distributions of 194 glass PIT-tag vertical distance scores for the Control, 20 and 40 atmosphere treatment classes combined.

7.3.2 PIP tag GLMs

None of the PIP experiment tags failed during the course of the experiment.

The interaction between 'Reading-event' and 'Treatment' was not significant in the mixed model GLM ($P < F = 0.7276$). In the main-effects GLM, 'Reading-event' was explanatory ($P < F = 0.0001$) but 'Treatment' was not ($P < F = 0.1081$). The Tukey test for differences in 'Reading-event' MVDS means revealed that initial 'Reading event' mean was significantly higher than subsequent 'Reading event' means (Appendix 6;
Figure 20).

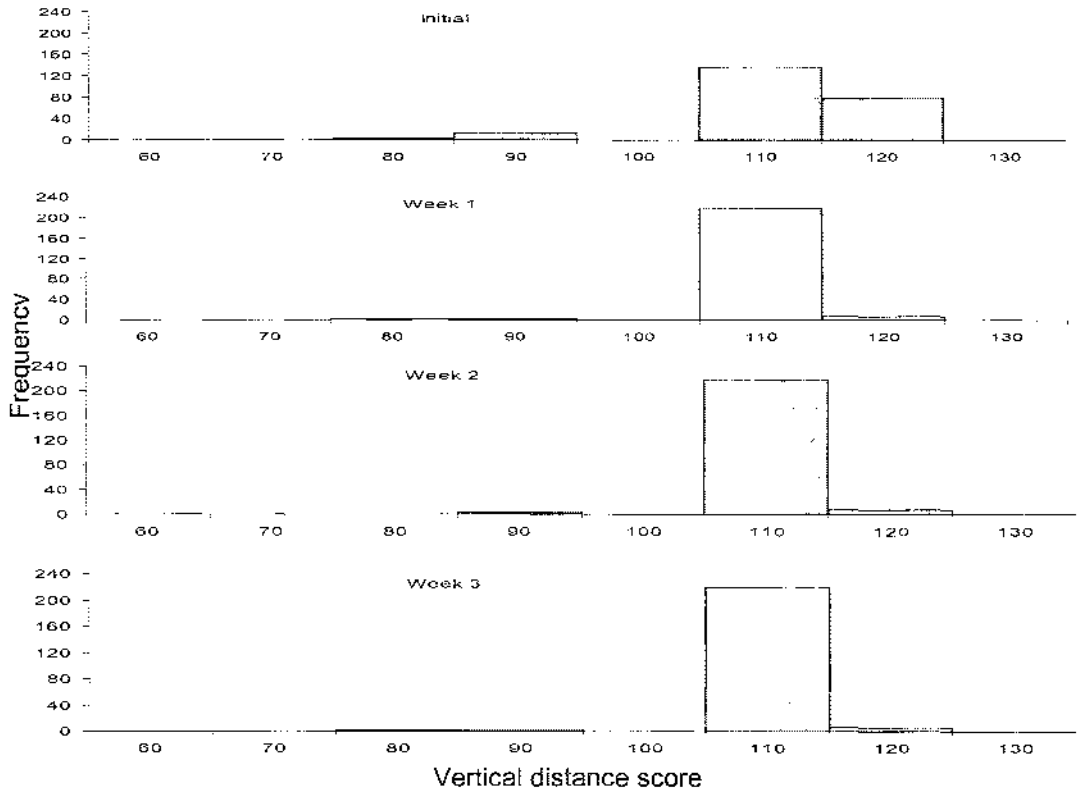


Figure 20: **Weekly frequency distributions of 229 PIP tag vertical distance scores for the Control, 20 and 40 atmosphere treatment classes combined.**

7.3.3 Effect of observed delamination PIP acrylic shells

After three weeks variable pressure exposure, 29% of the 40-atmosphere treated PIPs and 21% of the 20-atmosphere treated PIPs showed some evidence of oil intrusion under the acrylic shell (Table 13).

Table 13: **Number of PIP tags showing evidence of delamination between the acrylic shell and inner epoxy**

			Weeks in chamber		
	Delamination	Initial	1	2	3
20 atm	N	100	100	100	79
	Y	0	0	0	21
40 atm	N	100	100	100	71
	Y	0	0	0	29
Control	N	29	29	29	29
	Y	0	0	0	0

SEM examination of the tags revealed that the oil intrusions resulted from a delamination between the acrylic shell and the epoxy inner core (Figure 21). The delamination was confined to the acrylic-epoxy interface, and no physical breach of the epoxy medium had occurred. Similarly, no cracks were present in the acrylic shell.

Figure 21: SEM cross section through a delaminated section of a PIP.

The effects of 'Treatment' and 'Delamination' on the MVDSs were not significant under GLM analysis either as an interaction ($P < F = 0.0814$) or as main effects ('Delamination' $P < F = 0.7596$; 'Treatment' $P < F = 0.5505$).

7.3.4 Comparison of glass and PIP tag MVDSs

The effects of 'Tag type' and 'Reading event' failed to produce a significant interaction under GLM analysis ($P < F = 0.1761$). As well as 'Reading event', differences in the MVDSs were significant in relation to 'Tag type' as a main effect ($P < F = 0.0001$). Based on the MVDS the PIP tags performed marginally better than the glass tags. The aggregated mean MVDS for the PIP was 111.13 mm compared to 109.25 mm for the glass.

7.4 Discussion

None of the PIP tags failed as a result of pressure exposures of 20 and 40 atmospheres while immersed in fish oils. On the basis of the GLM results there was no evidence that the performance of the PIP tags, as measured by the MVDS, was impaired by 20 and 40 atmosphere pressure exposure for 3 weeks. Significant differences in the MVDS were found between the initial reading and the three subsequent reading events. As these differences were also reflected in the control PIPS, an explanation other than the effect of pressure exposure is likely to apply.

Delamination observed on some of the PIP tags at the time of final reading had no significant effect on the MVDS. This supports the assertion that the acrylic shell, although providing structural rigidity, is not required to protect the tag electronics against the ingress of fluids and gasses. The potential for slight liquid and/or gas intrusion into the acrylic and epoxy interface at pressures of 20 atmospheres is not grounds for concern.

There was no evidence that the PIP tag performance was worse than the glass tag under pressure exposure, and MVDS analysis suggests that the opposite is more likely.

8 RESULTS FROM POST 2002 SNA 8 TAGGING PROGRAMME PIP TAG EVALUATIONS

8.1 Introduction

During February and March 2002, 22 854 snapper were tagged with the 12 mm PIP tag and released off the west coast of the North Island. Over the subsequent 18 months, 1754 tagged fish were recovered from about 1000 t of commercially scanned catch.

The estimation of SNA 8 biomass based on the tag recoveries was presented by Gilbert et al (2005). Work undertaken in conjunction with the 2002 SNA 8 tagging programme, which has further relevance to evaluating PIP tag performance, includes a larger initial mortality study conducted exclusively on the 12 mm PIP in 2003 and tag recoveries from 2089 double-tagged snapper.

8.2 Results from a large scale trawl mortality study on the 12 mm PIP tag

8.2.1 Methods and Results

A more fully structured initial tag-mortality study was conducted on the 12 mm PIP tag in 2003. The basic bootstrap methodology was identical to that described in Section 2. The hypotheses tested was that initial mortality amongst PIP-tagged snapper is of a different order to that of CWT and Dart tagged snapper.

The bootstrap procedure adjusted for observed mortality amongst the experimental controls and was identical to that described in Section 2. However, the null hypotheses was two-tailed, meaning generated probabilities less than 2.5% or more than 97.5% constituted rejection at the 5% level.

Mortality amongst control snapper in the Dart/CWT studies was 2.0% (4 mortalities amongst 196 controls); in the PIP tag studies it was 10.2% (18 mortalities amongst 176 controls).

'Shot-weight' and 'fish-length' categories were arbitrarily chosen to maximise the number of classes while still maintaining a 'reasonable' number of cell observations (Table 14).

Table 14: Number of experimental observations across tag-type, shot and length.

Tag-type	Shot-weight (kg)	Fish-length (cm)					
		25 – 28	29 – 32	33 – 36	37 – 40	≥40	Total
CWT/dart	0 – 149	116	165	128	76	51	536
	150 – 299	82	111	73	60	43	369
	300 – 499	57	80	52	35	37	261
	500+	44	81	69	30	44	268
Total		299	437	322	201	175	1434
PIP	0 – 149	158	88	41	19	13	319
	150 – 299	38	91	72	43	15	259
	300 – 499	47	51	33	19	15	165
	500+	22	44	36	25	12	139
Total		265	274	182	106	55	882

None of the treatment cell comparisons were significant meaning that the null hypotheses could not be rejected at the 5% level for any cell (Table 15).

Table 15: Bootstrap adjusted mortalities for 12 mm PIP and CWT/Dart tagged snapper by treatment class; test of hypothesis that initial mortality amongst PIP-tagged snapper was of a different order to that of CWT and dart tagged snapper.

Length-class (cm)	Shot-weight (kg)	Adjusted Mortality*		
		PIP	CWT/dart	P[CWT<PIT]
25 – 28	0 – 149	0.352	0.447	0.919
	150 – 299	0.401	0.536	0.866
	300 – 499	0.667	0.566	0.123
	500+	0.649	0.622	0.328
29 – 32	0 – 149	0.345	0.260	0.071
	150 – 299	0.375	0.335	0.240
	300 – 499	0.334	0.293	0.255
	500+	0.429	0.628	0.965
33 – 36	0 – 149	0.308	0.221	0.105
	150 – 299	0.236	0.201	0.248
	300 – 499	0.326	0.207	0.080
	500+	0.373	0.581	0.956
37 – 40	0 – 149	0.283	0.180	0.111
	150 – 299	0.229	0.277	0.620
	300 – 499	0.143	0.251	0.707
	500+	0.502	0.423	0.210
40+	0 – 149	0.206	0.096	0.079
	150 – 299	0.181	0.137	0.208
	300 – 499	0.180	0.185	0.359
	500+	0.376	0.311	0.230

*bootstrap means adjusted for control fish mortality

The overall conclusion drawn from Table 15 is that the expected level of initial mortality amongst trawl caught and PIP tagged snapper did not differ significantly from that associated with other tag types.

8.3 An investigation of PIP tag failure using double-tagged snapper recoveries

8.3.1 Methods and Results

Of 22 854 PIP-tagged snapper released on the west coast in 2002, 2089 were double-tagged, i.e. two tags inserted into the peritoneal cavity. There were 147 recoveries of double-tagged fish. The second tag was missing from 15 of these fish. All tags recovered from the remaining 132 fish were functional. Although some failed tags may have been overlooked in fish where only one tag was found, the probability of this was low. Identified by the tag number, staff were aware at the time of tag recovery that a fish had been double-tagged, and therefore knew to look for the second tag. The more likely explanation for the missing double tag was that it was not in the fish to begin with. It was impossible to verify the presence of two tags in a fish at the time of release because the scanners would read only one tag of the double-tag pair. It was relatively common for the second tag to be withdrawn from the fish as the hypodermic was removed, and this probably occurred unnoticed on a number of occasions. Definitive evidence of tag failure would have been to recover a failed tag from a double tag pair, and this did not occur.

9 CONCLUSIONS

Two sizes of PIP tags were developed for potential use in the 2002 SNA 8 tagging programme (12 mm and 23 mm PIP). On the basis of live testing there was some evidence that the larger tag may induce marginally higher mortality in snapper than the small tag, but in most tests the two tags produced similar results. The overall result from live testing was there was no evidence that injection with a PIP tag produces any significant long-term deleterious effect in most of the snapper tagged.

Mortality levels associated with trawl capture, PIP tagging, and release were equivalent to levels observed for trawl capture and tagging with CWT or dart tags.

The 12 mm PIP was found to be robust against prolonged pressure exposure down to 40 atmospheres, and to chemical leaching or intrusion.

The observed lack of failure of any of the 132 double tag pairs of 12 mm PIPs recovered from released snapper indicates that their expected reliability factor for periods up to 2 years is better than 99%.

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Appendix 1: Number of snapper tagged by treatment class for initial mortality assessment

23 mm PIP			12 mm PIP	
Shot	Shot weight (kg)	tagged	Shot Weight kg	tagged
1	18.5	21	26.2	22
2	20.8	2	113.4	50
3	20.8	8	430.5	51
4	100.7	53	47.9	27
	Total	84	Total	150
Longline controls				
	Total	77		

Appendix 2: Main categories of histopathological lesions observed, and their lesion severity scores.

Lesion	Lesion severity score
Inflammation (normal)	0
Inflammation (heavy, unresolved)	2
Myodegeneration (focal)	0
Myodegeneration (extensive)	2
Inflammatory cell tracking (focal)	0
Inflammatory cell tracking (extensive)	2
Melanisation (focal)	0
Melanisation (extensive)	2
Fibroplasia (focal, resolved)	0
Fibroplasia (extensive, unresolved)	2
Hyperplasia (focal)	0
Hyperplasia (extensive)	2
Granuloma formation (resolved)	2
Granuloma formation (unresolved)	4
Haemorrhage (focal)	2
Haemorrhage (extensive)	4
Necrosis (focal)	2
Necrosis (extensive)	4
Bacterial present	4
Evidence of peritonitis/hepatitis	8
Fungi present	4
Evidence of mycosis	8
Myxozoa present	4
Evidence of protozoal disease	8

Appendix 3: Results for the grossly visible lesions observed (in parentheses) and pathological lesion score recorded from fish tagged with the 12 mm PIP tag.

Sex (M/F ratio)	*Benedenia* lesions	*Benedenia* on external surfaces	Tag entry site lesion score	Tag lodgment site lesion score	Liver lesion score	Total lesion score
2	2	2	0	0	2	2
2	0	0	0	6	0	6
2	0	0	0	0	4	4
2	0	6	0	2	0	2
2	2	0	0	0	0	0
1	0	0	0	2	0	2
2	3	8	0	2	0	2
2	0	8	10, (melanisation)	0	0	10
2	1	0	0	6	0	6
2	2	3	0	2, (tag in gonad)	4	6
2	4	4	0	0	0	0
2	7	8	0	2, (tag in gonad)	0	2
2	0	0	0	0	0	0
1	0	0	0	0	4	4
2	0	0	10, (melanisation)	0	0	10
2	2	3	0	0	4	4
2	3	7	0	0, (tag in muscle)	0	0
2	30	37	4	0, (tag in muscle)	4	8
2	2	2	0	6	0	6
2	0	2	0	0, (tag in gonad)	0	0
	55 %	60 %	15 %	40 %	30 %	75 %
1: 9	2.9	4.5	1.2	1.4	1.1	3.7
Sex ratio						
	0 - 30	0 - 37	0 - 10	0 - 6	0 - 4	0 - 10
	6.63	8.2	3.14	2.16	1.77	3.26

Appendix 4: Results for the grossly visible lesions observed (in parentheses) and pathological lesion score recorded from fish tagged with the 23 mm PIP tag.

23 mm PIP	Fish length (cm)	Sex (M/F ratio)	*Benedenia* lesions	*Benedenia* on external surfaces	Tag entry site lesion score	Tag lodgment site lesion score	Liver lesion score	Total lesion score
00204DA5	29	2	7	4	4	0	0	4
204D1C	29	2	0	0	0	0	0	0
00205163 * double tagged	31	2	0	2	0	12	4	16
00204E65	29	2	3	3	0	2	4	6
00204DCD	28	2	0	0	2, (slight ulceration at tag site)	0	0	2
00204DB1	26	2	3	10	0	0	4	4
00204E6C	26	1	3	0	0	0	2	2
005149C4	28	2	5	5	0	0	0	0
00204DD7	31	2	4	4	0	0	0	0
00204DD4	24	2	0	2	6	2	0	8
00204EOD	31	1	5	7	0	2	0	2
002057DB	28	1	2	2	0	2	0	2
0020568F	27	2	6	13	0	6	0	6
00204C7F	26	2	3	5	0	2	0	2
002057B5	26	2	1	2	0	2	0	2
00205686	24	1	6	6	0	2	0	2
00204DB8	29	2	0	1	0	0	0	0
00204DD1	32	2	3	0	4	0	0	4
00204E57	28	1	1	0	4	2	0	6
00204D18	26	1	3	1	0, (minor bruising)	2	0, (biliary stasis)	2
Prevalence			75 %	75 %	25 %	55 %	20 %	80 %
Mean abundance	27.9	1: 2.33 Sex ratio	2.75	3.35	1	1.8	0.7	3.5
Range	24 - 32		0 - 7	0 - 13	0 - 6	0 - 12	0 - 4	0 - 16
Standard deviation	2.29		2.24	3.54	1.89	2.82	1.49	3.72

Appendix 5: Results for the grossly visible lesions observed (in parentheses) and pathological lesion score recorded from control fish.

Control	Fish length (cm)	Sex (M/F ratio)	*Benedenia* lesions	*Benedenia* on external surfaces	Tag entry site lesion score	Tag lodgment site lesion score	Liver lesion score	Total lesion score
20984	29	1	1	1	0	0	0	0
00830	29	2	4	7	0	0	0	0
20153	30	2	0	0	0	0	0	0
00688	29	2	4	4	0	0	0	0
20935	26	1	1	0	0	0	0	0
01591	28	1	3	0	0	0	0	0
20816	26	2	3	3	0	0	0	0
00716	25	1	4	0	0	4	0	4
20811	28	1	5	8	0	0	0	0
21190	28	2	3	5	0	0	4, (biliary stasis)	4
Prevalence			90 %	60 %	0 %	10 %	10 %	20 %
Mean abundance	27.8	50:50 sex ratio	2.8	2.8	-	0.4	0.4	0.8
Range	25 – 30		0 - 5	0 - 8	-	1 - 4	0 - 4	0 - 4
Standard deviation	1.62		1.62	3.08	-	1.26	1.26	1.69

Appendix 6: Glass tag comparisons: Tukey's studentised Range (HSD) test on mean MVDS by 'Reading event'

Comparisons significant at the 0.05 level are indicated by '***'.

READ Comp.	Simultaneous Lower Confidence limit	Difference Between means	Simultaneous Upper Confidence Limit	Significance
1- 3	2.3531	3.6681	4.9832	***
1- 2	2.4186	3.7336	5.0487	***
1- 4	2.4841	3.7991	5.1142	***
3- 1	-4.9832	-3.6681	-2.3531	***
3- 2	-1.2495	0.0655	1.3805	
3- 4	-1.184	0.131	1.446	
2- 1	-5.0487	-3.7336	-2.4186	***
2- 3	-1.3805	-0.0655	1.2495	
2 - 4	-1.2495	0.0655	1.3805	
4- 1	-5.1142	-3.7991	-2.4841	***
4- 3	-1.446	-0.131	1.184	
4- 2	-1.3805	-0.0655	1.2495	

Tukey grouping	Mean	N	Reading event
A	113.325	194	1 (initial)
B	108.454	194	2
B	108.222	194	3
B	106.985	194	4

Appendix 7: PIP tag comparisons: Tukey's studentised Range (HSD) test on mean MVDS by 'Reading event'

Comparisons significant at the 0.05 level are indicated by '***'.

READ Comp.	Simultaneous Lower Confidence limit	Difference Between means	Simultaneous Upper Confidence Limit	Significance
1 - 3	2.3531	3.6681	4.9832	***
1 - 2	2.4186	3.7336	5.0487	***
1 - 4	2.4841	3.7991	5.1142	***
3 - 1	-4.9832	-3.6681	-2.3531	***
3 - 2	-1.2495	0.0655	1.3805	
3 - 4	-1.184	0.131	1.446	
2 - 1	-5.0487	-3.7336	-2.4186	***
2 - 3	-1.3805	-0.0655	1.2495	
2 - 4	-1.2495	0.0655	1.3805	
4 - 1	-5.1142	-3.7991	-2.4841	***
4 - 3	-1.446	-0.131	1.184	
4 - 2	-1.3805	-0.0655	1.2495	

Tukey	Grouping	Mean	N	Reading Event
	A	113.9301	229	1
	B	110.2620	229	3
	B	110.1965	229	2
	B	110.1310	229	4